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Morphometry And Morphology Of Fetal Stem Arteries Of Human Placenta In Hypertensive Diseases ("toxemia") Of Pregnancy

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MORPHOMETRY AND MORPHOLOGY OF FETAL STEM ARTERIES OF HUMAN PLACENTA IN HYPERTENSIVE DISEASES ("TOXEMIA") OF PREGNANCY

by

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To Rosa, Diego and my parents.

ABSTRACT

The present investigation was undertaken to study morphometrically and morphologically the fetal stem arteries of the placenta ir hypertensive diseases of pregnancy and correlate the changes in these arteries with syncytial sprout proliferation on the one hand and on the other with the maternal and neonatal data.

The morphometry of the fetal stem arteries was carried out on placentas of fifty hypertensive cases; fifty placentas of normal pregnancy and fifty of acute fetal distress group served as controls. Thus a total of 150 placentas was examined. In each case sixty arteries were evaluated; the diameter of the lumen and the total diameter of each artery examined were determined and recorded on devised tables, thus providing an index which expressed the lumen to whole artery ratio. These measurements were restricted to arteries of the 3rd order which had a calibre from 100 to 300 micra.

The statistical evaluation involved the analysis of the variance and covariance; the relation between variables was examined by computing Pearson correlations for all cases and for the hypertensive group alone.

A significant reduction in the ratio of the lumen to whole diameter was found in the fetal vessels of the hypertensive group as compared with both controls. The mean ratio of lumen to whole diameter of the fetal vessels differed between

regions of the placenta, the most marked being in the parachorial and the least prominent in the parabasal region in all, i.e., hypertensive and control placentas. No significant differences in the mean diameter ratios were found among subgroups of the "toxemic" cases, i.e., pre-eclampsia, essential hypertension, and renal disease associated with hypertension of pregnancy. These findings suggest that in many cases of placental insufficiency in "toxemia" the basic abnormality may be inadequate fetal circulation through the villi.

Reduction in the lumen to whole diameter ratios of the fetal arteries was associated with an increase in the number of syncytial sprouts, suggesting that similar factors may be influencing both. The other variables, i.e., maternal age, gestational age, placental weight, infant's weight at birth, Apgar score, proteinuria, blood pressure and urinary estrogens, analyzed in this study did not show a significant correlation with the lumen to whole diameter ratios of the fetal arteries.

The morphological changes in the fetal stem arteries appeared to affect the intima, the media and the surrounding stroma. The alterations of the intimal layer consisted of proliferation and thickening of endothelial and sub-endothelial cells leading to narrowing of the lumen. These lesions were conspicuous in the larger vessels (up to 300 μ). In the smaller ones (100-150 μ) the proliferation of the sub-endothelial cells was present but without much participation of the

endothelial cells in the narrowing of the lumen. Changes in the media included proliferation of smooth muscle cells and fibrous tissue, followed by degeneration of smooth muscle cells and vacuolation of the arterial wall. Other lesions, i.e., thrombi, thromboemboli, and arteritis, were present but only occasionally. In the villous stroma of the "toxemic" placentas the smooth muscle cells were oriented in a sigmoidal fashion, "connecting" the fetal arteries.

On the basis of the present observations, it may be concluded that the fetal arteries of the placenta play an important role in "toxemia" of pregnancy and their alteration may contribute to the placental insufficiency commonly found in these group of diseases. It would appear that the vascular changes were a secondary effect of a more basic and yet undetermined factor.

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I. INTRODUCTION AND PURPOSE OF THESIS

Hypertensive diseases ("toxemia") of pregnancy are still the main cause of morbidity and mortality in the gravid population and continue to be a serious and unresolved problem in modern obstetrics. Moreover, perinatal loss and mortality rate among infants born to "toxemic" mothers is almost twice that of infants born following normal pregnancy (Sims, 1970). Many theories have been proposed to explain the etiopathogenesis of this disease or diseases. Among others, to these belong the possible role of prostaglandins and their importance in the autoregulation of utero-placental blood flow (Speroff, 1975); the probable alterations in the renin-angiotensin system (Chesley et al., 1965; Talledo et al., 1968); and a disorder of coagulation that may be responsible for a widespread intravascular coagulation (Bonnar et al., 1971; 1975; Howie et al., 1971).

The fact that women with "toxemia" of pregnancy show a reduction in both choriodecidual and myometrial blood flow (Browne, 1958; Dixon et al., 1963) prompted the suggestion that uterine ischemia is one of the main causes of this group of diseases. Further support for this theory came from Cavanagh et al. (1974) who produced a condition similar to "toxemia" by narrowing the uterine arteries of pregnant primates. It has become increasingly apparent that the main pathological

features in "toxemia" are secondary to alterations in blood flow through the placenta and attention has been focussed principally on changes in the maternal circulation.

The morphological status of the utero-placental vasculature in "toxemia" of pregnancy has been assessed by light (Robertson et al., 1975), electron (De Wolf et al., 1975), and fluorescent microscopy (Kitzmiller and Benirschke, 1973). The lesions in the maternal arteries from the placental bed were first described in "toxemic" women by Hertig (1945) and extended by Zeek and Assali (1950) who named the lesion "acute atherosis" because of its resemblance to atheroma. These observations were later confirmed by others (Marais, 1962; Dixon and Robertson, 1958; Robertson, Brosens and Dixon, 1967; 1975; Brosens, Robertson and Dixon, 1972). However, the nature of the changes in the utero-placental vasculature and their implication in pathological ("toxemia", diabetes, fetal malnutrition) pregnancies as well as their role in utero-placental ischemia is still controversial. The fact that "toxemia" of pregnancy may occur in an extrauterine pregnancy (Baehler et al., 1975) shows that uterine ischemia may be only important when the uterus contains placental tissue.

The involvement of the placenta in this syndrome has been studied extensively in the past and numerous mechanisms by which a damaged placenta could initiate all the changes clinically associated with "toxemia" have been postulated. Changes interpreted as being secondary to utero-placental ischemia are present in a fairly high proportion of placentas from

"toxemia" of pregnancy. The histological feature supporting this ischemic status is the hyperplasia and proliferation of the villous syncytiotrophoblastic cells (Tominaga and Page, 1966; Alvarez et al., 1967; 1969). This change is presumably an attempt at repair and replacement of damaged syncytium and, as such, the degree of proliferative activity has been regarded as an approximate guide to the severity of the ischemia to which the placenta has been subjected (Aladjem, 1968; 1974). On the other hand alterations of the fetal stem vessels that seem to reflect changes in the fetal circulation in relation to "toxemia" of pregnancy seldom have been mentioned in the literature. The few existing accounts are those of Paine (1957) and Fox (1967); these authors contend that a fetal obliterative endarteritis is a common placental feature in hypertensive pregnancies. The thickening of these fetal vessels was not assessed by objective measurements to date. Moreover, it is uncertain from the literature whether this feature is unique for the group of hypertensive disorder of pregnancy or whether it may be observed under other pathological conditions, and whether there exists any correlation between the severity of these changes and the clinical consequences in the mother and infant.

The present study was undertaken to assess morphologically and morphometrically the fetal stem arteries of the 3rd order (Arts, 1961) of the placenta by the application of light and electron microscopy in selected cases of hypertensive diseases ("toxemia") of pregnancy, and in acute fetal distress and normal pregnancies as controls, in order to

assess the status of fetal vasculature and to correlate this with the degree of syncytial proliferation, and the maternal and the neonatal states. The purpose of this thesis is to report the results of the different studies undertaken with the above aim, in an attempt at contributing to the knowledge of the basic placental pathology in "toxemia" of pregnancy and its implications in the well-being of the mother and the fetus.

II. REVIEW OF THE LITERATURE

A. INTRODUCTORY REMARKS

For more than seven decades the fetus has been exonerated and the placenta has been increasingly incriminated in the pathogenesis of "toxemia" of pregnancy.

The fact that "toxemia" commonly causes clinical complications in hydatiform mole, a condition characterized by the presence of functional trophoblast and the absence of a fetus, has been considered supportive of the placental role in this condition. Of the many theories that have been proposed implicating the placenta as the cause of hypertensive disorders of pregnancy, only the presently most popular will be reviewed.

1. Placental Toxins, Autolysis and Infarcts.

Schmorl (1893) and other authors showed that placental cells entered the maternal circulation, and Veit (1902) and Ascoli (1902) followed that the so-called syncytiolysin on the placental cells produced a syncytiotoxin which required an antitoxin for its neutralization.

Hofbauer (1907, 1908) pointed out that the symptom complex of eclampsia was initiated by the post-mortem autolysis of the placenta. All subsequent hypotheses which attributed eclampsia to placental infarcts were based on this concept

of placental autolysis. Young (1914) suggested that the "toxemia" of pregnancy resulted from the absorption into the maternal circulation of toxins liberated from necrotic placental foci. He also noted that a partially separated placenta in antepartum hemorrhage was more likely to cause toxic symptoms than one that is not attached to the maternal circulation. The toxic products from the dead placental tissue and blood clot upon absorption into maternal circulation caused the clinical manifestations. Young and Miller (1921) later maintained that the occasional failure to detect placental infarction in cases of fulminating eclampsia was due to the fact that some of the changes were microscopic, but nevertheless responsible for the autolytic products in the maternal circulation. This hypothesis stimulated extensive studies of placental infarcts and their possible role in eclampsia.

Numerous data on such studies are available in the literature, but there is still no agreement on the subject whether the placental infarcts occur more commonly in eclamptic than in normal placentas. Some authors (Williams, 1900; Siddall and Hartman, 1926; Montgomery, 1931; Clements, 1934; Hill and Trimble, 1944; Browne, 1950; Shanklin, 1959) have persistently denied any particular association between placental infarcts and eclampsia. On the other hand, Bartholomew et al. (1932; 1934; 1936), Harer (1936), Falkiner (1942; 1950) and Maqueo et al. (1964), among others, reported that placental infarcts occur more frequently and are more numerous in placentas in pregnancies associated with maternal hypertension

than in controls.

2. Premature "Ageing" of the Placenta.

Since Tenney (1936) and later Tenney and Parker (1940) described an increase in severity and extent of syncytial degeneration in "toxemia" of pregnancy when compared with normal pregnancies, other authors have reached the same conclusion on the basis of histological evidence.

Observations such as those of Burstein et al. (1956), Thomsen (1955) and Paine (1957) indicated that there is an increase of fibrosis of villi, syncytial degeneration, deposition of fibrin, formation of infarcts, loss of the Langhans cell layer and endovascular sclerosis of the fetal stem vessels; these features were considered to be morphological indices of placental senescence appearing prematurely in "toxemia" of pregnancy. Further evidence supporting the concept of premature ageing has been presented by Wang and Hellman (1941) who observed a progressive decrease in oxygen consumption per gram of placenta towards the end of pregnancy, and in the studies of Wislocki and Dempsey (1946; 1948) who found histochemical and cytological evidence of advanced ageing of the syncytium in eclampsia. In view of the fact that the above changes are not increased in placentas from prolonged pregnancies, other authors (Shanklin, 1959; Fox, 1967) did not consider some of these as an expression of a simple ageing process.

In summary it may be stated that the placenta undergoes a progressive maturation of which some features are inter-

preted by many investigators as an ageing phenomenon. Whether the associated morphological changes that are accentuated in "toxemia" of pregnancy may be considered as primary "senescent" changes or are a reflection of placental ischemia (whatever its cause), is a question to which no firm answer may be provided at present.

3. Placental Ischemia.

Page (1948) suggested that a relative ischemia of the placenta may produce widespread injury to the trophoblast. The portion of the trophoblast that is not sealed off by the infarcts may contribute toxic substances, one of which may be the thromboplastin. Page attempted to support the above belief by experimental evidence. Ogden, Hildebrand and Page (1940) noted that experimentally produced acute uterine ischemia caused a delayed rise in blood pressure in pregnant but not in non-pregnant bitches. Sindram (1943a; b) and Bastiaanse and Mastboom (1949; 1950), in agreement with Page, found that after producing uterine ischemia in dogs the blood pressure only rose in pregnant animals. In their view, placental ischemia resulted in the placental formation either of a pressor substance or of some factors that in turn stimulated the hypophysis, the kidney or the adrenal glands to secrete such substance.

Bastiaanse and Mastboom (1950) sought to explain the low incidence of eclampsia in Holland during the war by assuming that the whole muscular tone of the pregnant women, including that of the uterus, was lowered as a consequence

of poor nutrition, and that the muscular hypotonia diminished the risk of placental ischemia. In 1933 Smith and Smith suggested that the placental ischemia was elicited by deficiency in estrogen and progesterone. The possible role of ischemia was studied by estimating the uterine blood flow in normal and during pre-eclamptic pregnancies (Browne and Veall, 1953; Assali et al., 1954; Morris et al., 1956; Moore and Myerscough, 1957; Taylor et al., 1958; Landesman and Knapp, 1960; and Smith, 1970). All the authors agree that there is a significant reduction of the utero-placental blood flow in pre-eclampsia. However, as pointed out by Page (1972), it is not known whether the reduction of flow preceded or followed the clinical syndrome.

Many of the placental changes in toxemia of pregnancy are considered to be secondary to ischemia. Among these, the thickening of the villus basal membrane (Tenney, 1935, 1936; Fox, 1964a), proliferation of syncytial knots (Alvarez and Benedetti, 1971) obliterative endarteritis of the fetal vessels (Ludmir, 1967), and proliferation of the cytotrophoblast (Wilkin, 1965) are most often mentioned. Some of these changes have also been reproduced experimentally in human placental explants by a reduction of the oxygen tension (Tominaga and Page, 1966; Fox, 1970; MacLennan et al., 1972). What causes the hypoxia à priori and whether more than one factor may be involved has not been definitely established to date. The "acute atherosis" affecting the maternal spiral arteries first described by Hertig (1945) in hypertensive states of

pregnancy and confirmed by others (Zeek and Assali, 1950; Marais, 1962; Brosens, 1964; Robertson et al., 1967) may be either directly or indirectly associated with insufficiency of deciduoplacental perfusion.

4. Hyperplacentosis.

Jeffcoate (1966) noted that in certain cases of "toxemia" of pregnancy the placenta was at times overactive as judged by size, histological appearance and gonadotrophin production, irrespective of parity and other factors. The author referred to this as "hyperplacentosis" and quoted as examples of this condition the hydatidiform mole, hydrops fetalis, multiple pregnancy and diabetes mellitus. Page (1972), in supporting Jeffcoate's hypothesis referred to the work of MacDonald's group in Dallas who found that pregnant women with the placental clearance of labelled dehydroisoandrosterone above the normal range frequently developed pre-eclampsia. However, as emphasized by Jeffcoate and Scott (1959), in many cases of "toxemia" of pregnancy the placenta does not show any sign of overactivity and, on the contrary, it is "small in size, often infarcted, and shows histological evidence of excessive trophoblastic degeneration".

5. Placental Antigens and Immunologic Implications.

Theories postulating that anaphylaxis and isoimmunization resulting from the placental transfer of fetal blood, proteins and metabolic products, play a causative role in hypertensive

disorders of pregnancy date back to the beginning of this century (Flexner, 1902; Dienst, 1905).

One recurrent theme in the literature has been the hypothesis that the inability of the primigravida to cope immunologically with the antigenicity of the fetoplacental unit might be an important factor in the development of pre-eclampsia (Scott and Beer, 1976). Interaction of maternal and fetal immunocompetent cells usually takes place at the choriodecidual junction of the placenta where the decidual vessels represent a potential area of imtimate contact. Kitzmiller and Benirschke (1973) have noted that the anti-gamma globulin and anti-complement fluorescent staining of the decidual vessels was positive only in women with pre-eclampsia; this suggested that immune reactions were occurring in these patients. Beer et al. (1972) were able to induce placental damage severe enough to cause abortion by injecting anti-placental serum in experimental animals. Other authors (Lanford et al., 1967; Smith et al., 1967; Okuda and Grollman, 1966) using anti-placental serum in experimental animals succeeded in eliciting a pre-eclampsia-like syndrome consisting of hypertension, proteinuria and decreased renal renin. Vardi and Halbrecht (1974) have described two placental antigens in placental connective tissues from pre-eclamptic patients which are not found in placentas from normal pregnancies.

It may be stated on the basis of the above that the role of immunologic factors in "toxemia" is at best circumstantial.

6. Endocrinological Implications of the Placenta.

The impact of the placenta upon the maternal endocrine glands results in the modification of their function.

With possible relevance to toxemia Smith and Smith described, almost 40 years ago (1933, 1940), an increase of chorionic gonadotrophin and a decrease of estrogens and progesterone in the maternal serum and urine.

Paine (1957) and Russell et al. (1957) noted that the degeneration of the syncytiotrophoblast was associated with a lowered excretion of pregnandiol. On the other hand, Jeffcoate et al. (1959) found a high gonadotrophin excretion associated with overactivity of the cytotrophoblast.

Sandler and Baldock (1963) have reported an increase of placental serotonin levels in pre-eclampsia. This increase has been attributed by them to ischemic placental injury with subsequent loss of monoamine oxidase activity.

Senior et al. (1963) showed that the placental metabolism of 5-hydroxytryptamine was reduced in pre-eclampsia, whereas the metabolism of tryptamine was not affected.

Nevertheless, the altered levels of any placental hormone may be only a consequence of a general derangement of the placental function, rather than factors initiating that derangement.

B. HISTORICAL NOTES

The origin of the term "eclampsia" has been the subject

of discussion and controversy in medical literature for a long time. Most authors state that "eclampsia" is derived from the Greek language and means "flash of light" or "shining forth", and was used by Hippocrates and Galen to describe convulsions in childhood and early adulthood. In 1682, Castelli defined "eclampsia" as "a brightness, lightning, effulgence of shining forth" referring to the writings attributed to Hippocrates (Castelli, 1682).

Dieckmann (1952) stated that "eclampsia" was mentioned in the ancient Egyptian, Chinese, Indian and Greek medical literature, without documenting his sources. He also wrote that Paulus of Ægina and Celsius mentioned epilepsy caused by the pregnant uterus.

During the Middle Ages the term "eclampsia" was applied to diseases accompanied by epileptiform seizures, e.g., "eclampsia infantum" and "eclampsia gravidarum". The differences between epileptic and eclamptic convulsions were discussed for the first time in de Sauvage's nosology published in 1763. De Sauvage also attributed the term "eclampsia" to Hippocrates and proposed the differentiation of "eclampsia" from epilepsy on the basis that the latter was a chronic disease while convulsions of acute causation characterized the former (Chesley, 1976).

Mauriceau (1668, 1694) published notable books referring to "eclampsia" as an obstetrical disease. Mauriceau stated that eclampsia was improved by the delivery of the fetus,

assuming that the condition might be, in many cases, due to toxic agents derived from the dead and decomposed fetus.

In the latter part of the eighteenth century Hamilton (1775) in his book of midwifery wrote: "No disease is more dreadful and alarming in appearance than 'convulsions'; though they are confined to no particular period of pregnancy they are most frequent and most dangerous in the later months".

In spite of the fact that most of the authors in the older writings emphasized the dramatic aspects of "eclampsia" there are only a few references to the entire spectrum of the disease, probably owing to the fact that, as pointed out by Chesley (1976), obstetrics was largely in the hands of midwives.

As mentioned above, "eclampsia" was not differentiated from epilepsy until 1739 (de Sauvage) and the distinction was not generally accepted for another century. Ryan (1831) recognized "eclampsia" as an entity, but later others, e.g., Churchill (1856), described the gestational convulsions as "manifestations of hystery or epileptic and apoplectic in nature". During the last part of the nineteenth century the increasing knowledge of the hepatic and renal lesions in this disease lead to the recognition of "eclampsia" as a distinct entity (Schmorl, 1893). Some of the clinical symptoms related to eclampsia were described independent of, and only later related to the disease. Thus, Mauriceau (1694) was one of the first to describe the presence of severe edema

in an eclamptic patient. Lever (1843) reported that he had found albumin in the urine in patients with puerperal convulsions and, even more importantly, he established the difference between eclampsia and the uremic phase of Bright's disease. The association between eclampsia and hypertension was delayed possibly owing to the lack of proper methods for measuring the blood pressure. Ballantyne (1885) was probably the first to suggest that in eclamptic women the arterial blood pressure was considerably increased. In 1903, Cook and Briggs observed that proteinuria was usually associated with hypertension and thought that the blood pressure was a better guide to prognosis than the proteinuria. The differentiation of "eclampsia" from renal diseases and essential hypertension has been relatively recent. Herrick et al. (1936) noted that essential hypertension was an important component of the hypertensive disorders of pregnancy. They also established the fact that what various authors termed a "chronic nephritis" during and following pregnancy was often, in fact, an essential hypertension.

Eclampsia has been designated properly by Zweifel (1904) as the "disease of theories". De L'isère (1850) in his book entitled "A Treatise of the Diseases and Special Hygiene of Females" summarized the main factors that were postulated during that century as being causative in the production of eclampsia. He at first pointed out that primigravid women with "sanguine temperament" and rachitic condition seemed

to be more liable to become eclamptic. Moreover, he noted that particular states of the atmosphere have also been among the predisponent factors. Finally he states: "The effects of imprudence in eating, and disorders in the exercise of other functions of the economy, the use of high-seasoned food, a depraved appetite frequently gratified, attacks of indigestion, the abuse of coffee, drunkenness, abuses of coitus especially in the later months of pregnancy, the abuse of alcoholic drinks, the impressions made by odours, the use of tight dresses or corsets, and in fine, the effect of the passions whether of joy or grief and all more powerful emotions of the soul" may predispose to "eclampsia". In the early years of the nineteenth century the "mechanical pressure theory" of the causation of convulsions was held generally. During the subsequent years the suggestion that the "eclampsia" was a consequence of a toxemia gradually assumed importance. The concept of "eclampsia" as a manifestation of a toxemia was probably first introduced by Smith (1849). Later, Murphy (1862) described that: "The direct proximate cause of convulsions is impure blood" and "Predisposing causes of convulsions are hyperemia, anemia and toxemia".

At the beginning of the twentieth century the almost unanimous opinion was that the disease was caused by a toxin. An enormous amount of work was carried out in an attempt at identifying it, albeit without a definite success.

Dixon and Taylor (1907) reported on the presence of a pressor activity in alcoholic extracts of placenta. However, Rosenheim (1909) believed that bacterial contamination could have caused the effect described by Dixon and Taylor and rejected their conclusions.

Young (1914) suggested that the placenta was the source of the supposed toxin. Impressed by the frequent occurrence of infarcts in the placentas of eclamptic women, he brought forward experimental evidence that the toxemia was due to autolytic products liberated in the early stages of the placental death. The concept of circulating toxins gained acceptance and physicians believed that the gravid uterus released toxic products into the circulation which produced a group of otherwise unrelated diseases classified as "toxemias of pregnancy". Included were such diverse conditions as acute yellow atrophy of the liver, hyperemesis gravidarum, Wernicke's encephalopathy, all forms of hypertension and other conditions. Kerr et al. (1933) stated that the subject of toxemia was teeming with opinions and theories such as autointoxication, complement "deviation" resulting from some "foreign" substance in the blood, anaphylaxis, endocrinological and deficiency stages, and electrochemical changes. In Midwifery by Ten Teachers (White et al., 1935) the authors discussed the various hypotheses of the causes of toxemia and indicated that the most popular theory was that of the direct or indirect transfer of toxins from the

placenta to the maternal circulation. According to these authors the toxins interfere with the efficient function of the liver and kidneys thus resulting in the faulty elimination of metabolic products. McIlroy (1936) pointed out that, whereas nobody had ever isolated a toxic substance from toxemic patients, the clinical manifestations were very similar to toxic poisoning by the snake venom. Schneider identified later (1947) the "toxin" in placental extracts free of particular matter, as a thromboplastin; this was quite a step ahead of the modern suggestion that eclamptic lesions may be the result of intravascular coagulation.

Most of these old theories have been disproved or discarded in whole or in part. Some, however, with certain modifications that bring them in line with the modern knowledge, merit reappraisal. Oliva (1944) made an attempt at summarizing the different periods of relevant research in "toxemia" of pregnancy. The author divided the research efforts into four periods: organic, immunological, physio-chemical and endocrinological. Organ-related explanations were offered until the end of the nineteenth century, and etiological factors were searched for in the liver, kidneys, intestines or in the fetus.

Immunological causes were suggested at the beginning of this century primarily in relation to reactions against the fetal or placental proteins.

Between 1910 and 1930 physio-chemical mechanisms were

implicated in the belief that metabolic phenomena could be affected by pregnancy. The search for endocrinological factors and metabolic changes took place largely in the period between 1930 and 1950. To the above four periods Rippmann (1976) added a fifth, i.e., the enzymological phase, which has been most popular since 1950. During the last 25 years countless biochemical studies have been carried out in search of postulated differences between the metabolic processes in the placenta from pre-eclamptic patients and those from normal pregnancies. New hypotheses have been introduced on the basis of observations or experiments that either could not be confirmed or were interpreted differently by various investigators. The pregnancy-induced hypertension remains a disease of unknown etiology.

C. NOMENCLATURE AND CLINICO-PATHOLOGICAL DEFINITION

Different terms in medical literature have been used to define and delineate the group of conditions denoting forms of hypertensive disorders of pregnancy. Rippmann (1976) compiled 60 different terms from the English and additional 40 terms from German literature alone. Since de Sauvage in 1793 coined the word eclampsia as a generic term for convulsions of acute etiology, this word was used indiscriminantly to include obstetrical and non-obstetrical conditions for almost 100 years. At the beginning of this century its use in relation to hypertension in pregnancy

became increasingly common and almost exclusive.

The term "toxemia" was employed in the belief that the gravid uterus was releasing toxic products into circulation which were producing a characteristic group of diseases. Stander (1929) questioned the use of this term on the basis that toxic substances actually were never isolated from toxemic patients. Vignes (1932) went further in calling for discarding its use, and finally in 1970 the American College of Obstetricians and Gynecologists officially declared the term "toxemia" to be a misnomer (Rippmann, 1976). Nevertheless, it still continues to be used rather often, largely because of lack of a better and all-embracing substitute. The obstetricians in Europe have been strongly in favour of using the term EPH-gestosis (E=edema, P=proteinuria, H=hypertension) to denote the entire group of hypertensive disease of pregnancy (Rippmann, 1971).

Over the past 100 years several classifications have been suggested for these diseases including at times disorders of entirely obscure and quite different nature. For example, Williams (1912) proposed a classification and in that included such entities as: "pernicious vomiting", "acute yellow atrophy of the liver" and "presumable toxemias".

The many classifications suggested prior to 1940 were essentially variations on Williams' scheme with a few modifications. Thus, Goodall (1936) divided acute pre-eclampsia into four clinical types: 1. renal, 2. hepatic, 3. hemorrhagic,

and 4. neural. He also differentiated between two forms of the renal type, in this group, i.e., degenerative and inflammatory.

In 1940, the All-American Committee on Maternal Welfare (Bell et al., 1940) published a classification in which the "pernicious vomiting" (see above) was retained but the "acute yellow atrophy" was omitted.

In 1952, another group, lead by Eastman, proposed a classification from which the "vomiting" was eliminated. They did not take into account the group of chronic renal diseases in the belief that these entities were differentiated from the pre-eclampsia and essential hypertension with ease. Nelson (1955) omitted the edema from the definition of pre-eclampsia and divided the disorder into a mild and a severe form, according to the degree of proteinuria present.

The Organization Gestose lead by Rippmann (1971) suggested the term EPH-gestosis and devised a new classification to encompass symptomatic and pathogenetic associations and concomitant diseases.

In 1974, a Committee of the American College of Obstetricians and Gynecologists recommended a new classification (Sullivan, 1974). This embraces four groups: 1. pre-eclampsia-eclampsia, 2. chronic hypertension of whatever cause, 3. chronic hypertension with superimposed pre-eclampsia and 4. late or "transient" hypertension. The last classifi-

cation is the most universally accepted at present.

The diagnostic criteria for this group of diseases have been vague and arbitrary in most of the systems of classifications devised, largely owing to the fact that clinically the different entities are often indistinguishable. Moreover, there have been problems with the definitions of hypertension and proteinuria as applied to these states of diseases, thus, further contributing much to the confusion and misunderstanding. For example, some authors have based their diagnosis of hypertension either on the presence of the systolic pressure of 140 mm Hg or more and diastolic pressure of 90 mm Hg or more, both to be demonstrated on two occasions with a minimum interval of six hours between measurements, or on an increase of 30 mm Hg or more in the systolic level, or 15 mm Hg or more in the diastolic level (with the same six-hour interval for both measurements) (McCartney, 1973). Others prefer to utilize only the mean arterial pressure in assessing the hypertension and consider the rise of 20 mm Hg as being significant (Page, 1972).

Traditionally, edema has been regarded as one of the main signs of "toxemia" of pregnancy. However, in the past few years some doubts have arisen regarding its use as one of the parameters in the analysis of the hypertensive diseases of pregnancy (Page, 1976). It is reasonable to assume that there is more than one cause for generalized edema of preg-

nancy just as there are multiple causes for proteinuria and hypertension. Moreover, as pointed out by Page (1953), edema may be present in almost every pregnant woman near term. The latter and the fact that generalized edema in pregnancy does not appear to be harmful to the patient or to her offspring (Vosburgh, 1976; Friedman and Fox, 1976) have brought some controversy to the use of this parameter for the diagnosis of pre-eclampsia, although edema is usually present in the course of the disease. Determining the presence and degree of proteinuria in the pregnant woman who has also edema and hypertension is very important in order to differentiate pre-eclampsia and eclampsia from other diseases in which a proteinuria may occur. Since the amount of protein found in the urine varies widely in health and disease, and the methods used by different authors also are not the same, it is uncertain what degree of proteinuria determined by which of the techniques employed should be regarded as being abnormal.

The method most often utilized to detect proteinuria is a semi-quantitative analysis of casually collected random specimens with results expressed as: negative, trace and from 1+ to 4+. Page (1972) quoting the recommendation of the American College of Obstetricians and Gynecologists defined proteinuria as "more than a trace" or, quantitatively, as 0.3 gm or more per 100 ml of urine. De Alvarez (1976) considered a 1+ in a specimen from a pregnant woman as

being significant and possibly indicating pre-eclampsia, especially in the presence of hypertension. He also pointed out that proteinuria is usually the last of the sequential manifestations to appear in the absence of a pre-existing renal disease. However, other authors (Friedman and Fox, 1976) believe that a 1+ proteinuria is not significant and proposed, instead, that the amount of 300 mg or more of protein in a 24-hour collection has an indicative value. Although there is a general agreement that minimal proteinuria is probably of no clinical importance, the issue of its level at which it may be considered significant, remains unresolved.

Whereas there is a general agreement that the pre-eclampsia-eclampsia is a disease of the second half of pregnancy, some controversy exists regarding the delineation of time of the onset of symptoms. Thus, in an Editorial of the C.M.A.J. (1972) pre-eclampsia was defined as a condition that develops after the 28th week of pregnancy, but some investigators (McCartney, 1973; Sullivan, 1974) regard the pre-eclampsia as a disorder that manifests itself after the 24th week of gestation. The latter delineation is widely accepted in today's literature.

D. THE TROPHOBLAST

The trophoblast constitutes the essential placental tissue which sustains growth and nutrition of the embryo by securing nourishment for it from the maternal circulation. Trophoblast produces placental hormones and at the same time is the site of active and passive transport of metabolites necessary for the fetal growth and development. Skinner (1961) defined trophoblast as follows: "(from) Greek: nourishment plus germ or sprout. The forerunner of the placenta or nourishing organ of the embryo. The name was given to this embryonic formation by Hubrecht in 1889". Hubrecht, a European embryologist, indeed coined the word trophoblast and thereafter (1889) elaborated upon its significance. He described it as follows: "The outer shell of the blastocyst is thickened and consists of three to four layers; it possesses honeycombed lacunae. For this outer (epiblastic) shell the name trophoblast may be chosen". He went on to say: "I propose to confer this name upon the epiblast of the blastocyst as far as it has direct nutritive significance, as indicated by proliferating processes, immediate contact with maternal tissue, maternal blood, or secreted material".

Langhans (1882) was the first to demonstrate that the cells covering the villi of the chorion frondosum and chorion leve were of fetal origin. He also showed that these cells formed two layers: a superficial "epithelial" layer and

a deeper "Zellschicht". The latter was termed subsequently by others the Langhans layer. As the superficial layer was demonstrated to be syncytial in nature it is usually known as the syncytiotrophoblast, and the Langhans layer, as the cytotrophoblast. In this communication the terms cytotrophoblast or Langhans cells and syncytiotrophoblast or syncytium will be used to conform with the terminology of the majority of the workers in the field.

While the mode of formation of the cytotrophoblast has been fairly well established the factors relating to the differentiation of the trophoblastic syncytium remain a matter of great controversy.

In the early stages of development the term blastocystic trophoblast has been widely used to denote the cells constituting the outer wall of the developing zygote. The primitive cytotrophoblast and syncytiotrophoblast are derived from the blastocystic trophoblast (Boyd and Hamilton, 1970).

1. Cytotrophoblast.

The primitive cytotrophoblast is derived from the cells of the wall of the blastocyst and forms all cellular and syncytial trophoblastic derivatives.

Boyd and Hamilton (1970) have defined several varieties of cytotrophoblast depending on their location and function. These include:

Villous cytotrophoblast (Langhans layer), constitutes the cellular or inner layer of the villi.

Cytotrophoblastic cell columns, arrange themselves to form peripheral "caps" on the tips of early tertiary villi and thus contribute to the elongation of the placental villi.

The columns never develop mesenchymatous derivatives equivalent to the mesenchymal cores of the villous stems of villi. By proliferating they form an irregularly arranged but an almost complete cytotrophoblastic layer known as the cytotrophoblastic shell.

Cells of the cytotrophoblastic shell, surround the whole zygote and later become part of the boundary zone of the basal plate and contribute to the interlobar septa and probably to the placental giant cells and to the peripheral syncytium.

Cells of the cytotrophoblastic island, apparently originate from those of the cell columns.

Cells found in the lumina and wall of the spiral arteries of the decidua, in the early weeks of pregnancy cells that proliferate from the cytotrophoblast of the conceptus, overrun the basal decidua and invade the decidual portion of the spiral arteries. By the end of the second trimester these changes may involve even the endings of the maternal radial arteries (Robertson et al., 1975).

The specific characteristics of the villous cytotrophoblast possesses the important function of a germinal layer which, in addition to regenerating itself for a certain period, gives rise to the syncytium.

Morphologically, the cytotrophoblastic cells have definite characteristics which distinguish them from the overlying syncytium. Their shape may vary from ovoid to polyhedral and the variation in size reflects their frequent division (Wislocky and Bennett,1943). The cytoplasm of these cells has been described as clear (Wislocki and Bennett, 1943), and much less basophilic than that of the syncytiotrophoblast (Boyd and Hughes, 1954). The pale basophilic staining of the cytotrophoblast has been correlated with the presence of only a few profiles of endoplasmic reticulum (Boyd and Hamilton, 1970).

On the basis of light microscopic observations, Wislocki and Bennett (1943) suggested that the cytoplasm of the cytotrophoblastic cells may be surrounded by a delicate membrane or capsule which may be visualized after applying Mallory's connective tissue stain. However Boyd and Hamilton (1966) using electron microscopy demonstrated that the Langhans cells do not possess such capsules, and attributed the above findings as artifacts of staining.

Ultrastructurally, the Langhans cells show a "simple" organization: large nuclei with prominent nucleoli, large mitochondria, a few profiles of Golgi apparatus, microfibrils, desmosomes and a well defined basement membrane (Wynn, 1975; Enders, 1965a; Boyd and Hamilton, 1970). Despite the fact that most of these ultrastructural characteristics are those of undifferentiated cells the primary function of which is growth rather than elaboration of specialized endocrine or

exocrine products, some features are not those to be expected of stem cells. To these belong the presence of junctions between adjacent cytotrophoblastic cells, desmosomes, and well developed microfibrils.

The presence of desmosomes between the Langhans cells and the syncytium has been considered as general evidence for the close relation between both types of the trophoblastic cells, and as an indication that the syncytium is derived from the cytotrophoblast (Enders, 1965b). A basement membrane is known to separate the cytotrophoblast from the villous stroma (Boyd and Hamilton, 1970; Ashley, 1965; Panigel, 1974). The superficial portion of this basement membrane has been regarded as the glycocalix of the Langhans cells (Boyd and Hamilton, 1970; Liebhart, 1974); it is presumed to play an important role in cellular transport (Groniowski, 1968; Groniowski et al., 1969), and in the cellular immunological mechanism (Billington, 1975). The trophoblastic basement membrane increases in thickness through and, especially, towards the end of pregnancy (Tenney, 1935; Hall, 1949).

The fate of the Langhans cells has been a matter of debate and controversy. For many years, the classical histologist had reported that they are absent in the later part of pregnancy. It was believed by some (Froboese, 1924) that this layer "disappears" as early as in the third or fourth month, and by others (Stöhr, 1959; Ortmann, 1949; 1955) that this occurs in the fifth or sixth month of pregnancy. However,

many investigators have showed that the Langhans cells never totally disappear and may be found in full term placentas either as isolated cells (Boyd and Hamilton, 1970), small groups of cells (Panigel and Anh, 1963), or even as an almost continuous layer (Wislocki and Bennett, 1943; Baker et al.,1944; Sauramo, 1961; Wigglesworth, 1962). Mitoses may be found frequently in the cytotrophoblast and centrioles may persist until the end of gestation in the residual cells of Langhans layer. In some cases the cytoplasm of these persisting Langhans cells may show vacuolation and frank degeneration (Boyd and Hamilton, 1970).

2. Syncytiotrophoblast.

The term syncytium was introduced approximately one century ago to indicate the multinucleated masses of protoplasm found in certain animal tissues.

The origin of the trophoblastic syncytium and its subsequent growth have been disputed for a long time. However, continuing evidence for the derivation of the syncytium from the cytotrophoblast has been provided on the basis of several different observations (Florian, 1928; Brewer, 1937; Hertig and Rock, 1941).

Bargmann and Knoop (1959) suggested that the multinucleated placental cytoplasm represents in fact a plasmodium which arise not from coalescence of originally separated cells but from a mass of multinucleated protoplasm. These observations, however, have nct been confirmed by others

and at present there is a general agreement that the syncytial trophoblast represents a multinucleated mass of protoplasm established by the fusion of cells (Rhodin and Terzakis, 1962; Terzakis, 1963; Pierce and Midgley, 1963; Carter, 1964; Wynn and Davies, 1964; Enders, 1965a; b; Boyd and Hamilton, 1966; Okudaira et al, 1966).

By light microscopy the syncytium of the chorionic villus is seen as a layer of syncytioplasm largely without cell boundaries. In the Hematoxylin-Eosin preparations the nuclei of the syncytium, when present, are described usually as being small, irregular and strongly basophilic (Wislocki and Bennett, 1943). The basophilia has been attributed to the presence of the ribonucleic acid (Singer and Wislocki, 1948) and associated with the protein synthesis (Wislocki and Padykula, 1961). The nuclei tend to be situated in cytoplasmic regions "away" from the intervillous space. It is now generally accepted that the mitotic division occurs frequently in the cytotrophoblast but is not present in the syncytial nuclei. Some authors have postulated that the division in the syncytium occurs by amitosis. This view was supported on the basis of the occasional presence of dumbbell-shaped nuclei (Florian, 1928) or of two elongated nuclei lying side by side and being V and Y shaped (Johnston, 1941). Apparently, the Y-shaped nuclei represented the first, the V-shaped nuclei the second and the side-by-side nuclei the third and complete phase of the process of division.

To distinguish the possible amitotic nuclear proliferation within the syncytium from cytotrophoblastic origin of the syncytiotrophoblast, Galton (1962) used microspectrometry based on the measurement of nuclear deoxyribonucleic acid (DNA). His findings led to the suggestion that the rapid accumulation of nuclei in the syncytium may be explained by cellular proliferation within the cytotrophoblast followed by coalescence of daughter cells into the syncytium. Other authors (Tao and Hertig, 1965; Richart, 1961; Pierce and Midgley, 1963) on the basis of morphological studies utilizing autoradiographic techniques concluded that synctium was being formed from cytotrophoblastic cells.

The gradual disappearance of the Langhans cells without evidence of their degeneration in great numbers has been offered as an additional support of the contention that the cytotrophoblast covering the villi is largely transformed into syncytium (Boyd and Hamilton, 1970).

One of the interesting features of the early trophoblast is the presence of the vacuoles in the syncytium (Streeter, 1920; Florian, 1928; Johnston, 1941; Hamilton and Boyd, 1960; Wislocky and Bennett, 1943). The vacuoles are variable in size in different areas of the villous system, and persist in some regions until late in pregnancy. The "foaming" and vacuolation may be interpreted as reflecting a constant movement of the syncytioplasm and generally is considered to indicate that in the living state the syncytioplasm is plastic, pleomorphic, and capable of changing its shape

(Boyd and Hamilton, 1970). Wislocki and Bennett (1943) believed that the syncytial vacuoles perhaps were a manifestation of increased fluid absorption by the embryo. By light microscopy, a delicate vertical striation, a so-called brush-border may be observed in some regions of the syncytial villous surface. This brush-border was first described by Kastschenko (1885) and its presence was later confirmed by some authors (Fellner, 1903; Schaffer, 1927; Boyd and Hamilton, 1970) and denied by others (Nagy, 1960). A detailed description of the placental brush-border based on light microscopic study was provided by Wislocki and Bennett (1943), who stressed also its great variability. In some regions of the chorionic villi no definitive brush-border could be identified. On the basis of their study these investigators concluded that in the living state the trophoblastic syncytium showed a considerable instability. Moreover, they suggested that the various surface irregularities of the syncytium were involved in the uptake of the fluid and nutrient materials from the intervillous space.

Transmission electron micropscopy (and more recently scanning electron microscopy) have more accurately defined the microvillous configuration of the syncytiotrophoblast and its variable ultrastructural appearance.

Studies by different authors (Boyd and Hughes, 1954; Wislocki and Dempsey, 1955; Boyd and Hamilton, 1970) showed that the trophoblastic syncytium is not a simple passive

membrane. Areas of different ultrastructural appearance have been described by Burgos and Rodriguez (1966). They have described two main areas designated as an alpha and a beta zone. In the alpha zone a thin layer of the cytoplasm faces the endothelium of a fetal capillary apposed to the basement membrane of the trophoblast covering of the villus. The close vicinity in this region of the maternal and fetal circulation favors the simple diffusion phenomena, e.g., an exchange of respiratory gases. Microvilli are numerous at the apical border of the syncytium as a micropinocytotic vesicles, dense bodies and dilated cisternae of smooth endoplasmic reticulum, all indicating that in this area absorptive function and active transport predominate (Panigel, 1974). In the beta zone the presence of highly developed profiles of rough endoplasmic reticulum, and a conspicuous number of filaments and lipoid inclusions implies that this area is associated with a high secretory activity and protein synthesis. A third area of the syncytiotrophoblast has been described (Dempsey and Luse, 1971) as the "basal accrual" zone which is close to the cytotrophoblastic cell; the organelles in this area closely resemble those of the Langhans cells.

The multivesicular bodies that are present in the syncytium have been interpreted as elements that may function in the selective hydrolysis and transport of proteins (Panigel, 1974). In the last four months of pregnancy, there are extensive regions where the syncytium is much attentuated.

Some workers have suggested that these regions are involved in the transport across the trophoblast (Panigel, 1974). Bremer (1916) described thin parts of the villi devoid ofsyncytial nuclei. These areas were particularly obvious over stromal capillaries and were called by him "epithelial plates". Subsequently, Getzowa and Sadowsky (1950) suggested the complex: the "epithelial plates", the basement membrane of the syncytium and of the capillary endothelium, and the endothelial cells themselves, all constitute the so-called vasculo-syncytial-membranes. Apparently, these membranes are of considerable functional importance in the feto-maternal transfer of gases and metabolites.

In addition to the regional variations in thickness several other regional variants of the trophoblastic covering layer have been described.

Since some aspects of the present work involved the assessment of synctial proliferation this phenomenon will be reviewed in some detail as it applies to placentas of normal and "toxemic" pregnancies.

3. Syncytial Proliferation in Normal Placenta.

Different specialized regions of the trophoblast other than the already described "epithelial plate" have been observed over the years. Considerable confusion arose from the terminology used by different authors in connection with those areas in which there is an aggregation of syncytial nuclei. The terms syncytial sprouts, masses, buds, knots

and syncytial globules, and nuclear clumps and proliferation nodes, have been interchangeably used (Duckworth, 1908; Getzowa and Sadovsky, 1950; Alvarez et al., 1964; Fox, 1965). A classification based on the different types of specialization of the trophoblastic syncytium of the chorionic villi has been proposed by Hamilton and Boyd (1966). They have defined at least three types of syncytial specialization other than the "epithelial plate":

Syncytial sprouts, are projections from the syncytial surface into the intervillous space;

Syncytial buds, are projections from the syncytial surface into the stroma of the villi;

Syncytial clumps or knots, are thickened areas of the syncytium often adjacent to the epithelial plates in which nuclei are massed or grouped together.

a) Syncytial Sprouts. Syncytial sprouting may occur as early as the twenty-fourth day after implantation (Boyd, 1956). The degree of syncytial sprouting in early pregnancy has been considered as one of the most important signs of normal placental activity (Alvarez, 1964). As pregnancy progresses the wide syncytial sprouts seen at the time of implantation become vascularized and give rise to the terminal and preterminal villi.

The existence of the syncytial projections was first recognized by Kastschenko (1885). Subsequently, the sprouts

were described as accumulations of nuclei within a definite and circumscribed area, at the side of the villus or, in other cases, at its prolongation (Duckworth, 1908). The same author stated that at approximately the ninth week of pregnancy the syncytial sprouts are less conspicuous than earlier, and some of those present have an attenuated peduncle. Studies carried out by Alvarez (1964) by phase-contrast microscopy had shown them as cylindrical, club-shaped or pseudopodium-like, sessile or pedunculated masses of cytoplasm without cellular delineations and containing a large number of nuclei of different sizes. The same investigator observed that the number of sprouts diminished as the pregnancy advanced.

Other authors had indicated that there are, in fact, two varieties of sprouts (Hamilton and Boyd, 1966). To the first variety belong those present in the initial phase of the development of the free villous and are only stages in the formation of new chorionic villi. They are usually covered by a cytotrophoblastic lining; cells from the stroma of the villus forming a core and vascularization of this core complete the process of what, in effect, is the production of a new villus branch. The second variety of syncytial sprouts does not have either the cytotrophoblastic layer or the stromal core. Moreover, the stalk of attachment joining them to the villous system becomes attenuated. Sometimes they detach and become ovoid masses of syncytium of up

to 500 μ or more in diameter, floating freely in the intervillous space. These free syncytial sprouts may enter into the maternal circulation from the intervillous space and this phenomenon had been termed "the deportation of trophoblast" and has been a matter of great controversy. It was first described by Schmorl in 1893. He found masses of such trophoblast in the lungs in 14 of the 17 examined women dying of eclampsia, but not in the lungs of four patients dying of other causes. In a later study, Schmorl (1905) confirmed his previous observation as did also other investigators (Veit, 1905; Park, 1958; Bardawil and Toy, 1959).

The regular presence of syncytial sprouts in the blood of the uterine veins and the inferior vena cava of living women during pregnancy was first described by Douglas and co-workers (1959) and confirmed by other authors (Thomas et al., 1959; Thomas, 1961; Ikle, 1961; 1964). However, until now trophoblastic sprouts have only been found in vessels between the uterus and lungs (Boyd and Hamilton, 1970).

Morphologically, the trophoblastic sprouts must be distinguished from the maternal megakaryocytes (Minot, 1922), atypical hemoblastic cells and osteoclasts (Alexander and Spriggs, 1960) which, like the sprouts, are multinucleated. However, as pointed out by Boyd and Hamilton (1970), all the former cells do not possess a brush-border which characterizes the trophoblast. The fate of the trophoblast in the

maternal lung has been discussed extensively in the literature, but this subject is not within the scope of the present review.

The number of syncytial sprouts at three different levels of the fetal cotyledon (basal, intermediate and chorial) was assessed by Alvarez et al. (1970) in normal placentas at term. They reported that the number of syncytial sprouts is lowest at the basal plate, increased at the intermediate plate, and is highest at the chorial plate. At the marginal lake the number of sprouts is even greater than that observed at the chorial plate. These findings prompted the authors to conclude that the gradient of trophoblastic proliferation at different zones of the placenta depends upon the pO_2 gradient; thus, the blood with the highest pO_2 is present in the intracotyledonary cavity, while the lowest pO_2 is found at the marginal and the subchorial lake. The same authors (Alvarez et al., 1969) also calculated that the number of syncytial sprouts in the normal placenta of 38 weeks' (or more) gestation does not exceed 8 percent of the villi.

b) Syncytial Buds. Boyd and Hamilton (1964) were the first to describe the consistent presence of small spherical masses of trophoblast within the stromal cores of the villi under the term of "stromal trophoblastic buds". Already in 1960 Ishizaki had observed what appeared to be similar

changes in the stroma of the villi, but in this case the trophoblast involved was calcified and according to his description, was associated with a placentitis. Ishizaki termed these changes "orphan bodies" and discarded the possibility that there is any connection between these structures and the layer covering the villi. Other authors had also observed the presence of syncytial buds, the so-called "syncytial globules" (Alvarez et al., 1964), or "bourgeons trophoblastiques a l'interieur du stroma villositaire" (Wilkin, 1965). The morphological appearance of the syncytial buds was extensively described by Boyd and Hamilton (1964) and Hamilton and Boyd (1966). These "buds" had an outer covering layer of cytotrophoblast which represented the part of Langhans layer invaginated into the villous stroma by inwardly projecting syncytium. Fibrinoid, nuclear pyknosis and other degenerative changes were observed within the substance of these buds.

The functional significance of the syncytial buds, if any, is not known with certainty. The possibility that by their breakdown the buds may be liberating hormones which could have an influence on the fetus has been suggested (Boyd and Hamilton, 1964). The presence of the trophoblastic syncytium in the umbilical cord blood at the time of delivery (Salvaggio et al., 1960) suggested to Boyd and Hamilton (1964) the possibility that the buds, or at least their syncytial contents, could make their way through the endothelium into the fetal blood vessels and from thence pass into the fetus.

However, in 1970 the same authors (Boyd and Hamilton) without discounting the possibility of the presence of trophoblast within the fetal vessels under normal conditions regarded the regular passage of such buds into the fetal circulation as an uncommon feature.

c) Syncytial Clumps or Knots. The syncytial knots were defined as being circumscribed conglomeration of the syncytium containing clumped nuclei and present largely in the terminal villi in areas adjacent to the epithelial plates (Hamilton and Boyd, 1966). Various theories have been proposed with regard to the origin of the syncytial knots. Some authors (Tenney, 1936) believe that these represent a degenerative phenomenon whereas others interpret them as a manifestation of an ameboid activity of the syncytium (Baker et al., 1944). Getzowa and Sadowsky (1950) dismissed the theory that the syncytial knots are the product of a degenerative process. Instead, they had proposed that these structures are normal constituents in term placentas, usually related to the development of the so-called "vasculo-syncytial-membrane" (see above).

Further evidence in support of this theory was presented by Alvarez (1964). Some authors had suggested that syncytial knots form bridges between adjacent villi to protect the epithelial plates from the changes of blood pressure in the intervillous space (Hörmann, 1953).

The concept of syncytial knotting as a manifestation of syncytial proliferation that results from a normal or an increased activity of the cytotrophoblast has been endorsed by several investigators (Shanklin, 1958; Alvarez, 1964; Fox, 1965) and denied by others (Vokaer et al., 1958). It has been observed that in the normal placenta the number of syncytial knots increases with gestational age, reaching the maximum in the mature placentas (Wigglesworth, 1962; Fox, 1965; Hamilton and Boyd, 1966; Alvarez et al., 1967; Aladjem, 1967). The last author using phase-contrast microscopy, had noted no apparent morphologic differences between the syncytial buds, knots, and the "migrating" trophoblast found in the maternal or fetal circulation.

Ultrastructural studies (Strauss and Okudaira, 1967) have shown that the knots are "related" to the proliferation and fusion of cytotrophoblastic cells in their course of differentiation into the syncytium.

A considerable controversy exists with respect to the factors that may be involved in the syncytial knot formation. Fox (1965) had proposed that their formation was stimulated by a reduced fetal villus blood flow, whereas Thomsen (1955) suggested a reduced maternal blood flow as the main cause. Studies carried out <u>in vitro</u> in which syncytiotrophoblast was exposed to a hypoxic environment have demonstrated that there was clustering of both syncytial nuclei and cytoplasm at one pole of the villus (Tominaga and Page, 1966). The

authors interpreted the above changes as a slow adaptation of the placenta to chronic hypoxia which results from circulatory impairment.

Significant variations in the distribution of the trophoblastic knotting were reported by Fox 1964a. Analyzing different zones of the placenta he found a lower number of syncytial knots in the intermediate zone of the placenta as compared with those present in the maternal and subchorial lakes. The percentage of the syncytial knots as an index of placental maturity has been proposed by some authors (Benirschke, 1961; Merril, 1963; Fox, 1965); it is considered that in the normal term placenta approximately 30 percent of villi show the presence of knots.

4. Trophoblastic Proliferation in "Toxemia" of Pregnancy.

The first published observation concerning the association of syncytial proliferation with "toxemia" was reported in the literature at the beginning of this century. In 1908 Brindeau and Nattan-Larrier described excessive growth of the syncytiotrophoblast which formed sprouts in relation to the blood of the intervillous space in placentas from eclamptic patients. In 1930, Riviere described an irregular hypertrophy of the syncytium in "toxemia" alternating with masses of "free plasmodia" in contact with the blood of the intervillous space. Subsequently, a series of placental studies in seventeen eclamptic and one hundred pre-eclamptic "toxemic" cases were presented by Tenney (1936). The

placentas of the patients with definite eclampsia showed 90 to 100% of the villi with degenerated syncytium; in the pre-eclamptic group degenerative changes were present in 50-100% of the villi. He also noted that between 10 and 40% of villi from normal pregnancies showed degenerated syncytium. In 1940, Tenney and Parker have confirmed the earlier findings and correlated these positively with the levels of albuminuria. They stressed that the essential placental lesion of eclampsia consisted of a premature ageing of the placental villi. Parenthetically, the characteristics of the "ageing" placental villi were described by Wislocki and Dempsey (1946) and later confirmed by Paine (1957). The former authors observed in eclamptic patients an abnormal rise in both acid and alkaline phosphatases in the cytoplasm of the syncytiotrophoblast and gradual diminution in cytoplasm of the nucleo-protein manifested as a loss of basophilia in such villi. Sauramo (1951) noted that in toxemia the syncytial knots were numerous and largely in the process of disintegration. Moreover, she found that in the chronic toxemia the syncytial changes were more marked than in the acute form of the disease. However, other authors (Burstein et al.,1957a) did not notice a difference between the degree of the placental changes and the severity of the disease although the placentas with the highest counts of syncytial knots were in cases with severe symptoms. Similar observations were reported by Nesbitt (1958), Shanklin (1959) and Merril (1963). Perhaps the first comprehensive attempt at

studying the syncytial proliferation by evaluating separately the syncytial knots and the sprouts was that of Alvarez (1967). Using light and phase-contrast microscopy he observed that the increased syncytial proliferation in "toxemia" of pregnancy was largely owing to the increase in the number of syncytial sprouts, whereas, the number of syncytial knots remained unchanged. Subsequently, the same author and co-workers (Alvarez et al., 1969; Alvarez and Benedetti, 1971) found a positive correlation between the levels of the arterial pressure and hyperplasia of both the cyto-, and syncytiotrophoblast. These authors point out that the trophoblastic hyperplasia should be interpreted as a consequence of the relative hypoxia produced by a reduced placental blood flow. They also showed that the number of syncytial sprouts in "toxemia" increased from the center to the periphery of the cotyledon, being more marked in the parachorial than in the parabasal and intermediate zones (Alvarez and Benedetti, 1971; Alvarez et al., 1972). The authors interpreted the marked proliferation of trophoblast in "toxemia" as an ability to respond to hypoxia, contradicting thus the theory of others that the "toxemic" placenta is an "aged" organ. Aladjem (1968) noted that there was an apparent similarity between the curve of excretion of the Human Chorionic Gonadotrophin (HCG) and syncytial sprouting in normal and abnormal pregnancies; the HCG excretion was elevated in patients with pre-eclampsia.

The proliferation of cytotrophoblastic cells as a result

of placental ischemia had been demonstrated experimentally by McKay et al. (1958) and Fox (1964b; 1970). The latter reported that no evidence of increased knot formation could be demonstrated in the "toxemic" placenta, and that the cytotrophoblastic proliferation present in this group of placentas was a "repair-hyperplasia phenomenon" as a result of ischemic damage to the syncytium (Fox, 1964b, 1970).

E. FETAL VESSELS OF THE PLACENTA

The gross anatomy of the human placenta is virtually a description of its vascular anatomy since it is a structure almost wholly composed of fetal blood vessels which have as their main function the presentation of the fetal blood to the maternal circulation.

The vessels of the human placenta were described in considerable detail, but it will become obvious from the following review that much remains to be learned regarding the precise anatomy and morphology of these important structures.

1. The Chorionic Vessels.

The fetal vasculature of the placenta is conveniently subdivided into vessels of the fetal surface or chorionic vessels and those of the maternal surface or cotyledonary vessels (Table 1).

The transitional region between the umbilical cord and the chorionic plate is largely formed by a perimuscular

collagenous layer of the umbilical vessels (Scheuner, 1964). Upon entering the chorionic plate the two arteries divide to supply branches to the cotyledons. Shordania (1929) described two main groups of the arteries in all human placentas according to the pattern of subdivisions and their branchings. He used the terms "disperse" and "magistral" respectively to describe these two forms. In the disperse pattern the two arteries divide several times dichotomously into a number of smaller vessels with gradually diminishing caliber. In the magistral pattern the two arteries give off only small branches in their course, and extend almost to the margin of the chorionic plate before their caliber diminishes. Shordania believed that the magistral placenta should be advantageous for the fetus, and that the vascular pattern was genetically determined by the maternal vascular characteristics.

As a rule each main umbilical artery begins to divide prior to reaching the placenta and the divisions usually are not of equal size. The "fate" of each of these primary divisions varies considerably from one placenta to another.

Each primary division undergoes further ramifications into vessels of second order, vessels of the third and sometimes even of the fourth order (Crawford, 1956). In the course of branching of the umbilical artery, the so-called perforating arteries arise and penetrate the chorionic plate to become the stem arteries of the central cotyledons of the placenta. At the marginal zone the arteries also perforate

the chorionic plate becoming the stem arteries of the peripheral cotyledons (Boyd and Hamilton, 1970). One dichotomous branch of a chorionic vessel of the second or third order may immediately pierce the plate and enter the cotyledon, whereas the other branches continue in the plate and divide into chorionic vessels of higher order. The umbilical vein is formed by the union of sub-divisions and divisions of veins having a course and distribution similar to their arterial counterpart. The veins of the main branches accompany the corresponding arterial vessels and their total diameter "matches" those of the arteries (Crawford, 1956).

2. The Cotyledonary Vessels.

The placenta is regarded as being composed of lobes which may be readily observed on its maternal aspect, and impart upon this surface a characteristic appearance. Each lobe in turn is made up of several cotyledons in close contact with each other. The cotyledon is the basic structural unit of the placenta and each is derived from a primary trunk arising from the maternal surface of the chorion. It is the division of this primary trunk and the subsequent divisions which gives each cotyledon its characteristic branched appearance.

Conflicting opinions are still held on the structure of a cotyledon and the distribution of its vascular pattern

(Table I). At the end of the last century, Bumm (1890) suggested that the fetal vessels enter the placenta through large stems from which the chorionic villi protrude. Some of these stems are fixed to the basal plate as the so-called "anchoring villi" whilst the branches of others terminate freely in the intervillous space. Spanner (1935) in his studies based on corrosion preparations disagreed with Bumm's theory. According to him the two umbilical arteries give rise to numerous placental arteries which spread fanwise in the chorionic plate. From these radial trunks, vertical branches are given off which follow the stem villi from the surface to the base of the placenta. In turn, each of these arteries divides into a numer of recurrent branches which penetrate the corresponding villi. These branch and re-branch to break up finally into the sinusoidal capillary bed of the smallest villi, which by "floating" freely is being "bathed" by the maternal blood. Spanner himself compares the distribution of these vessels with an old-fashioned chandelier. The venous blood is returned from the terminal villi through a system of veins which accompanies the arteries. According to Spanner the veins possess a series of muscular sphincters which act as checks upon returning venous flow. Similar findings were reported by other authors (Falkiner, 1939). However, Stieve (1940; 1941) rejected the theory of the free villi suggesting that the tertiary villi do not "float" freely but form instead a

communicating network. The fetal blood flows through the vessels in the chorionic tissue forming this net. Moreover, he suggested that the syncytium also forms a communicating network. Romney and Reid (1951) using the plastic injection method observed, at variance with the chandelier concept proposed by Spanner, that only those vessels of the main stem or anchoring villi that supplied the zone adjacent to the decidual plate reverse their course with a suggestive chandelier-like configuration. The capillary units, regardless of location, appeared to be short in contradistinction to Spanner's proposal that there was a relatively long capillary bed connecting with venous channels at the base of the villus. They also determined that whereas in the allantochorionic vessels the veins are approximately twice the diameter of the arteries no difference in diameters was found between arteries and veins beneath the chorion. The main stem arteries and veins appeared to be of the same calibre.

Based on his extensive work in which he used the method of injection with dyes, Bøe (1953) described the vascularization of the early and mature placenta. In the latter the chorionic tree in each cotyledon consisted of one main stem (truncus chorii) which ramified into major stems and their branches (rami chorii) including the anchoring stems and minor stems (ramuli chorii). The majority of the terminal branches and chorionic villi emerged from the minor stems. Bøe also stated that terminal branches and

villi are vascularized by two means: partly by branches from the stem vessels and partly from the superficial capillary network.

Based on his work in which he used a new technique of injection and corrosion, Wilkin (1954) described an organizational pattern of the mature placenta. The fetal cotyledon was shown to be supplied by a single artery and drained by a single vein from the chorionic plate. The artery in each of these was accompanied by the vein (first order blood vessels). As the first order vessels reach the cotyledon they divide into a number of secondary vessels (second order blood vessels) and these divide once again into the third order blood vessels. The villi in which the third order blood vessels lie are the anchoring or stem villi. Wilkin (1954) likened the shape of the cotyledon to a drum (tambour). In addition, he noted that in the center of the cotyledon there was a circular gap (couronne). Wilkin's concept of the fetal cotyledon has been accepted by others (Crawford, 1962; Freese, 1966; Reynolds, 1966; 1967).

The cotyledonary arteries and veins of the trunci, the rami and ramuli of the villous tree were categorized by Arts (1961) as representing the first, second and third order vessels, respectively. According to Arts, the caliber of the vessels perforating from the chorionic plate into the truncus chorii (major villus stem), now called truncal vessels, is 1.5 mm for the artery and 2 mm for the vein. The truncal artery divides

into cotyledonary branches of the second order with an average diameter of 1 mm. They curve in the direction of the basal plate to form the villous branches of third order or choriodecidual vessels in the ramuli chorii. Similar findings were described by Boyd and Hamilton (1970). On the basis of studies of the normal human placenta by microangiography (Krohn, Ivemark and Salo, 1970) two types of intracotyledonary arteries were distinguished. The first, type A, was defined as a long and narrow artery running straight from the chorionic area towards the decidua. The caliber of these arteries ranged from 100 to 250 μ. Their branches were of small caliber, always ran within the same stem and were usually arranged in the direction of the main artery. The second, type B, was thicker than type A, twisted and ranged from 250 to 500 μ in diameter. The branches of these arteries left the stem at a right, but occasionally at an acute, angle to the main artery. On the basis of this arterial pattern the authors classified the cotyledons into three groups: those with only type A arteries, those with only type B arteries, and those with both types of intracotyledonary arteries. They also identified a fourth cotyledonary group composed of an irregular mixture of different types of arteries.

3. Normal Structure of the Fetal Vessels in the Chorionic and Subchorionic Plate.

Whereas there is extensive literature on the subject

of topography of the placental vasculature, few contributions were made since the classical histological study of Eden (1897) concerning the actual structure of the fetal vessels and the variations to be expected to exist at the various segments of the vascular tree.

Thus, Eden (1897) had noted that in the chorionic stems and branches the arteries and veins showed three coats: the muscular coat consisted of longitudinal and circular fibres; the intima showed the endothelium and a very thin stratum of connective tissue, and the adventitia was composed of concentric rings of loose fibrous tissue which merge with the surrounding stroma. The author mentioned that there is no elastic lamina of either type present in these vessels. Bøe (1953) found that when the arteries leave the umbilical cord and enter the chorionic plate they partially lose their muscular coat. Simultaneously, the diameter of the vessels increases considerably and the arteries and veins have a similar appearance.

The structure of the fetal vessels was described in great detail by Burstein and co-workers (1956). They noted that, as elsewhere in tissues, in the placenta the arterial as well as venous channels of all sizes were lined by a single layer of endothelium. The PAS-positive fibrils forming the subendothelial layer were observed in all vessels but were more marked in the arterial than in the venous system. Usually, there was no subendothelial connective tissue in either the arteries or the veins. The media con-

sisted of circularly disposed muscle fibers which appeared hypertrophied and were arranged compactly. They found this muscular arrangement in arteries of all caliber, including "arterioles". At times, the lumina of the vessels appeared narrowed owing to the hypertrophy of the muscular layer rather than to an intimal thickening. The occasional presence of collections of connective tissue cells and/or PAS-positive substance which separated muscle fibers was also noted. The authors concluded that the veins could be distinguished from the arteries only by their thinner muscular layer.

During the development of the placenta, the arterial walls become thickened by proliferating connective tissue (Becker, 1963). On entering the chorionic plate and the trunci chorii there is a marked diminution of the extent of muscular thickness of the arteries involved (Bøe, 1953; Boyd and Hamilton, 1970). In agreement with other authors (Romney and Reid, 1951), Boyd and Hamilton also noted that the difference in diameter between arteries and veins observed in the chorioallantoic vessels was no longer apparent beneath the chorion, making it difficult to distinguish one vessel from the other.

Nikolov and Schiebler (1973) reported that characteristically the vascular wall shows numerous myoendothelial junctions and consists of many muscle cells in close contact with one another. At the end of pregnancy the vascular

endothelium and the smooth muscle cells exhibit all morphological criteria of full functional activity. The authors, confirming the early findings of Eden (1897), pointed out that in the umbilical arteries there is an ill-developed elastic layer whereas a special tunica elastica does not exist in placental vessels.

4. The Pathology of Fetal Stem Arteries.

The first published observation on the occurrence of abnormal changes in fetal vasculature of the placenta was reported in the literature in the nineteenth century. Fränkel (1873) described an "obliterative endarteritis" of the fetal stem arteries in placentas from women with luetic infection. He noted that the vessels were tightly enclosed and compressed by proliferating cells and explained this phenomenon as a "granulative proliferation of the cells".

In Germany, the fetal arteries were linked to the process of placental infarction.

Several investigators (Cohn, 1888; Ackerman, 1891; Eden, 1897) reported that the fetal vascular changes were the initial step in the formation of an infarct and that this lead to a "coagulation-necrosis" first of the epithelium and subsequently of the villous stroma. One of them (Ackerman, 1891) postulated that the initial change in the vessels is secondary to a reduction of pressure in the arterial and venous systems of the placenta with reactive thickening

of the intima, i.e., as it may occur in an inadequate cardiac activity of the fetus. On the other hand, Cohn (1888) and Franque (1894) explained the changes in the vessels as a result of a maternal infection, i.e., endometritis or nephritis. Some authors (Franque, 1894; Frankel, 1873) considered these changes in placental fetal vasculature as a possible reason for intrauterine death. However, Merttens (1894) believed that these vascular changes were secondary to fetal death since the placentas retained for the longest time showed quantitatively and qualitatively the most accentuated alterations. On the basis of a detailed study of the physiological and pathological features of the mature placenta, Eden (1897) suggested that some of the changes in the arterial wall were the result of a post-mortem retraction of the muscular coat; thus these were most marked in arteries that possessed the thickest muscular coat. At the same time he ascribed the presence of an endarteritis and periarteritis, and the proliferation of the muscular and subendothelial layer as representing an ageing process of the placenta. In an artery thus affected the obstruction was often completed by thrombosis or in other cases the lumen was entirely "obliterated" by the intimal thickening. The obliteration of extensive arterial tracts could produce an arrest of the circulation in large areas of the villi.

Williams (1900) was of the opinion that the primary cause of an infarct formation in the great majority of cases

was to be found in an "endarteritis" of the vessels of the chorionic villi. This theory of the etiology of placental infarction remained deeply rooted in the medical literature during the first half of this century (Bartholomew and Kracke, 1932; Hunt et al., 1936; Bartholomew and Colvin, 1938). Several reasons for believing that the nourishment of the villous syncytium was derived from the maternal intervillous, rather than from the fetal circulation, were presented by Young (1914). He opposed the theory that alterations of the villous vessels produced a placental necrosis and attributed practically all infarcts to changes in the decidual vessels. Other investigators (Siddall and Hartman, 1926; Strachan, 1926; Montgomery, 1931) were in agreement with the view of Young, failing to find the process of "endarteritis" in the placentas with infarctions except in those specimens where the placenta was the site of syphilitic disease. In a study designed to compare the placentas of syphilitic and "toxemic" patients, Riviere (1930) noted that the placental vascular changes were prominent in both diseases. Some vascular changes considered to underlie placental infarcts were attributed to hypercholesterolemia. Thus, Bartholomew and Kracke (1936) in a study of 1000 placentas observed acute infarcts in those from patients suffering from pre-eclampsia and eclampsia, and theorized that placental infarcts resulted from a cholesterol-induced arterial disease. Subsequently, Patterson

and co-workers (1938) performed total thyroidectomies on pregnant rabbits and found hypercholesterolemia in the fetuses. The vessels of these placentas were affected by severe cholesterol "endarteritis", probably predisposing to thrombosis of placental vessels and an infarction, resulted from a fetal hypercholesteremia, the latter being secondary to maternal hypothyroidism. The same authors (Hunt et al., 1940) noted in a study of 180 patients with different degrees of "toxemia" that an "endarteritis", sclerosis and perifibrosis of the placental vessels were present in the majority of the cases. However, they stated that unless these changes were extensive they probably did not lead to degeneration.

Paine(1957) described fetal vessels with medial thickening and endarterial proliferation, which often lead to obliteration, in placentas from pregnancies associated with essential hypertension and with pre-eclamptic "toxemia". He suggested that the changes were compatible with premature ageing of the placenta and related these to a possible reduction of functional placental bed in the area, especially in cases of essential hypertension. Other authors (Burstein et al.,1957a) denied than an alteration of the fetal stem vessels could be found in "toxemic" placentas, showing that the ratio of the lumen to the over-all diameter in the arteries was essentially the same as in placentas of normal pregnancies.

In the excellent review on the pathology of the fetal stem arteries, Fox (1967), in agreement with Paine (1957), noted two lesions occurring in the human placenta: an endothelial proliferation and a fibromuscular sclerosis. The former was found in placentas from uncomplicated pregnancies but were more common in placentas of cases of pre-eclamptic "toxemia", essential hypertension or diabetes mellitus. The author found generalized fibromuscular sclerosis only in placentas from stillbirths, probably as secondary post-mortem changes. He interpreted the endothelial proliferation as a result either of the fetal hemodynamic changes owing to the placental ischemia, or of an immunological reaction in the vascular wall.

Bender et al. (1976) described the presence of "endangitis obliterans" of the placental blood vessels in 73 placentas out of 4600 different pregnancies, including those complicated by proteinuria and hypertension. They considered that "endangitis obliterans" represented a form of placental insufficiency and pointed out that the etiological factors involved in this vascular lesion are multiple, whereas the morphological and patho-physiological reactions are the same. A fetal "obliterative endarteritis" was described also in placentas from cases of rhesus incompatibility (Burstein and Blumenthal, 1962; Burstein et al., 1963; Wilkins, 1965), in diabetic mothers (Burstein et al., 1957b; 1963; Fox, 1965), in relation to retardation of fetal growth (Gruenwald, 1961;

Koenig, 1972), in cases of heavy smoking during pregnancy (Lohr et al., 1972), in placentas from prolonged pregnancies (Thomsen and Schniewind, 1961) and from cases of premature onset of labour (Nezelof and Roussel, 1954). However, Driscoll (1965), and Wilkin (1965) were unable to find any arterial alterations in the placentas from diabetic mothers, and Fox (1967) found no significant alterations of the fetal vasculature in placentas from cases of rhesus incompatibility. On the other hand, Fujikura and Benson (1964) in studying 73 placentas of stillborn infants identified a complete fibrous occlusion of the fetal vessels in the stem villi of 28 placentas (38 percent). They described the obliterative process as a growing out of fibrous tissue from the subintimal area rather than an intimal proliferation, and considered this lesion as an antemortem phenomenon secondary to placental inflammation.

Similar changes were described in placentas from stillbirths by Becker and Dolling (1965) who regarded them as the cause of the intrauterine fetal death; they also assumed that maternal infections between the second and third trimester were the cause of the vascular obliteration. Wilkin (1965) studying 36 placentas from pregnancies terminating in stillbirth reported the presence of endothelial proliferation that led to complete obliteration of the lumen in the stem vessels of 1st and 2nd order. However, Fox (1967) found no evidence of endothelial proliferation after

fetal death. Instead, he believed that the vessels were almost occluded by a growth of fibrous tissue into the vascular lumen and he termed this a "fibromuscular sclerosis". The author also observed that all placentas from fetuses dead in utero for over a week showed severe lesions, whilst a placenta from a stillbirth that was retained only for a brief period showed a "mild" lesion. He did not find a relation between the lesion and a maternal infection, and believed that the vascular sclerosis is a post-mortem change. Different vascular changes in placentas following an intrauterine fetal death were reported by Theuring (1968). He described the presence of a reticular type of an obliteration of the vessels pointing out that the placentas of stillborn infants who were more macerated at birth showed the most severe changes with complete obliteration of the vessels. Other investigators (Koenning et al., 1972; 1973) studying placentas from high risk pregnancies have also described narrowing of the vascular lumina in the fetal placental circulation owing to a proliferative process. They suggested that by a certain stage these vascular changes may produce a chronic placental insufficiency and eventually fetal death.

Experiments in guinea pigs (Delaquerriere-Richardson and Valdivia, 1967) and rats (Emmrich et al., 1975) showed that the effects of total cessation of fetal blood flow that follow the fetal death produce progressive obstruction of the larger intraplacental vessels by proliferating intimal

cells leading in some cases to necrosis of the corresponding subplacental territory.

III. ORIGINAL OBSERVATIONS

A. MATERIALS AND METHODS

The placenta was collected in a prospective study of 500 consecutive "high risk" (Table 2), and 50 normal pregnancies. The group of "high risk" pregnancies was identified and selected according to a program of screening and diagnosis followed at the St. Joseph's Hospital, London, Ontario. In this group of "high risk" pregnancies special investigations, including systematic hematological and hormonal studies, uterine growth balance, placental transfer test, fetal electrocardiography and amniotic fluid analysis were carried out whenever necessary. In addition, a scoring system was used to predict perinatal mortality (Effer, 1969). This score included pathological factors, function tests, the vital statistics and a complete physical examination of the patient.

Of the placentas collected and processed, 50 placentas of hypertensive diseases of pregnancy, 50 placentas from acute fetal distress group and 50 placentas of normal pregnancies were selected for morphological and morphometric studies with special reference to the fetal vasculature and syncytial proliferation; the two last groups served as controls.

The maternal and neonatal data were obtained from clinical records; the variables controlled in the analysis were: maternal age (M.A.), gestational age (G.A.), placental weight (P.W.), infant's weight at birth (B.W.) and the Apgar score (A.S.) at 1 minute and 5 minutes. In addition to the variables analysed for all cases, values for proteinuria (P), blood pressure (B.P.), and the urinary estrogens (U.E.) were recorded for the hypertensive group. Three maternal conditions were distinguished in the hypertensive group: thirty six mothers were pre-eclamptic, eight had essential hypertension and six had chronic renal disease.

1. Collection and Processing of the Placentas.

The placentas were collected at the St. Joseph's Hospital within 24 hours following delivery, placed in plastic bags and stored at 4°C. The maternal and fetal surface of each placenta was photographed and examined grossly. Following inspection the membranes and all blood clots were removed from the placenta, the umbilical cord was cut off 5 cm from its insertion, and the placenta was weighed. Data pertaining to the gross examination (including weighing and measurements) were entered on the standard data sheets (COLR-3222-1,7-59; Collaborative Research, Perinatal Research Branch, NIH, Bethesda, Md.). Subsequently, the placentas were cut perpendicularly to the basal plate into slices

measuring approximately 1 cm in width. All slices were visually examined and whole-thickness blocks for microscopic examination were taken from the marginal and central parts of the placenta, the umbilical cord, membranes (sausage-shaped roll); and from macroscopically visible lesions. Only the block from central placenta was evaluated in this study.

For light microscopic examination the tissues removed in the fresh state were fixed in 10% buffered formalin solution for 24 hours and for the same additional time in a fresh identical fixative. After fixation the tissues were processed by the method of tetrahydrofuran (Haust, 1959), embedded in paraffin and sectioned at three to five micra. The sections were stained routinely with hematoxylin-phloxine-saffron (HPS), Periodic Acid Schiff (PAS), Resorcin fuchsin stain for elastica and Masson's trichrome. Other stains were used in addition whenever indicated.

For electron microscopy, tissues from selected fresh placentas were fixed for 90 minutes at 4°C in 3 percent buffered glutaraldehyde. Following washing in 0.1 M phosphate buffer (pH 7.4), small pieces of tissues (1 to 2 cubic millimeters) were postfixed for 90 minutes at 4°C in a solution of 1 percent osmium tetroxide in 0.1 M phosphate buffer (pH 7.4), and for an additional 30 minutes at room temperature. Dehydration of tissues was commenced in 50 percent ethanol and completed in four successive changes of ascending concentrations of ethanol. Tissues were embedded

in Epon-812 according to the method of Luft (1961). One-micron-thick sections were cut with glass knives on either Porter-Blum Mt-1 or Reichert ultramicrotomes and stained with alkaline toluidine blue for light microscopy. Thin sections were cut with a diamond knife, mounted on unsupported copper grids and stained doubly with uranyl acetate (Stempack and Ward, 1964) and lead citrate (Reynolds, 1963). Thin sections were examined with a Philips EM-300 electron microscope. In the present study only the fetal stem arteries of the 3rd order measuring 100-300 micra in diameter were studied (Arts, 1961).

Some fresh selected placentas were subjected to injection by latex solution (Ward) diluted 50% with distilled water for gross demonstration of the vascular tree (arteries in blue, veins in red) and documented by colour photography (Fig. 1).

2. Determination of the Number of Syncytial Sprouts.

For the determination of the number of syncytial sprouts, a reticle screen (Wild) attached to the head of the microscope was used (Fig. 2). One stained section of tissue removed from the central part of each placenta was divided into three layers of approximately equal width (Fig. 3) and parallel to the basal plate: the parabasal layer, adjacent to the basal plate; a parachorial layer, close to the chorial plate; and the third, the intermediate layer, between the

above two (Alvarez et al., 1970). The number of sprouts in 400 villi were counted separately for each area and were entered on an appropriately devised record table.

The syncytial sprouts were recognized as conglomerations of normal nuclei of variable size embedded in a cytoplasmic matrix which had no apparent cellular limits. They were attached at the end of, or laterally to the villus and appeared as free or pedunculated syncytial masses. For the purpose of this investigation only the sprouts attached to the villi were taken into account (Figs. 4-8).

3. Morphometry of the Fetal Stem Arteries.

For morphometry of the fetal stem arteries, a standard microscope reticle (Barrington, N.Y.) with the scale of 100 parts in 1 mm was placed in the ocular of the microscope. The same slide with its three subdivisions used to determine the number of sprouts was utilized to assess also the fetal arteries. In each zone of the three layers twenty arteries were evaluated as follows. The diameter of the lumen and the total diameter of a cross-section of the artery were measured and recorded on a devised record table, and an index was obtained which expressed the ratio of the lumen to the whole diameter. These measurements were limited to arteries of the 3rd order (Arts, 1961); the caliber of these is 100 to 300 micra (Fig. 9).

Selective examination and mensuration of arteries leads to an erroneous ratio which usually tends to be too low, as the observer has a natural tendency to select vessels with thick, well-formed media. With these reservations, the lumen to whole diameter ratio was found to be fairly constant for any particular case.

4. Definitions and Data for Statistical Analysis.

The "high risk" pregnancy is defined as "any pregnancy associated with a definable risk factor that increases the perinatal mortality or severe morbidity above normal".

The term pre-eclampsia was applied to a condition of a woman more than 24 weeks' pregnant who developed hypertension, proteinuria or pathological edema.

In analysing blood pressure we have employed the concept of mean arterial pressure (MAP) (Page, 1972), since it is considered physiologically the most important determinant. A MAP of 105 mm or greater was defined as "hypertension".

Proteinuria was defined as "more than a trace" or, to be more quantitative, as 38 mg or more per 100 ml of urine as recommended by the American College of Obstetricians and Gynecologists (Hughes, 1972). A gradation of 0-4 + was used. The recognition of a group with essential hypertension was based on the presence of a raised blood pressure prior to 24 weeks of pregnancy and evidence of hypertension persisting thereafter.

All the patients with a serious chronic renal disease who could be recognized by clinical investigation were clustered together in a third group.

Fetal distress has not been adequately defined in the literature. However, in clinical medicine it is usually recognized as a slowing of the fetal heart under 100 or acceleration beyond 160 beats per minute, and/or the presence of meconium in the amniotic fluid in all but breech presentations (Berendes, 1975). Both above parameters of the fetal condition, monitored during delivery were present in the group included in this study.

The normal group was represented by normal women who delivered their normal infants between 38 and 42 weeks of gestation.

Gestational age was expressed in weeks, considering the mother's last menstrual period.

The Apgar score of the infant at 1 minute and 5 minutes was determined by the attending nurse in consultation with the attending obstetrician.

The urinary estriol estimations were obtained within 5 days of delivery in nineteen of the fifty hypertensive pregnancies. All estimations were carried out in the laboratories of St. Joseph's Hospital.

The information obtained from the counts of syncytial sprouting and the assessment of fetal vessels in the placenta together with selected obstetrical and pediatric data

(M.A., G.A., P.W., B.W., A.S., P., B.P., U.E.,) were recorded on computer cards. The statistical evaluation involved the analysis of covariance and of variance. The relation between variables was examined by computing Pearson correlations for all cases and for the hypertensive group alone.

The analysis of covariance was used to determine the effects of the type of pregnancy on sprout counts and the ratios of the lumen to the whole artery diameters.

The analysis of variance was used to determine the effects of each area of the placenta after removing the effects of the type of pregnancy. For this analysis the data for each slide (one case) was divided into the three established layers and the mean for each area represented the basic value that was analysed. The design may be viewed as a partially nested design with subjects nested within the type of pregnancy and latter crossed with an area of the placenta. Correlations between the means of the seven variables established for all subjects (S.C., D.R., M.A., G.A., P.W., B.W., A.S.) and the means of the ten variables obtained in the hypertensive disease group only (S.C., D.R., M.A., G.A., P.W., B.W., A.S., P., B.P., U.E.) were analysed and are included with p- values (α =).

B. RESULTS

1. Syncytial Proliferation and Morphometric Studies of the Fetal Arteries.

a) Introduction. The three major objectives of this analysis were:

1. To examine the effect of the type of pregnancy("toxemia"), fetal distress,normal) on the sprout proliferation and fetal arteries of the placenta.

2. To test whether the sprouts and vessels differed from one placental area to another in the three groups.

3. To investigate the relation between the sprout counts, arterial diameter and the other variables recorded in this study.

The statistical techniques used under 1. and 2. were the analysis of covariance and the analysis of variance. The relations between variables (3) were examined by computing Pearson correlations for all cases as a pool and for the "toxemic", fetal distress and normal groups separately. Some of these relations were also evident in the analysis of covariance.

b) The Effects of the Type of Pregnancy on Sprout Counts and Lumen to Whole Diameter Ratios. There are some factors, not controlled in the design, that could affect the variables of response (sprout counts and lumen to whole diameter ratios). These are maternal age, gestational age, placental weight, birth weight and the Apgar score. The means of these variables as they occur in the three groups ("toxemia" of pregnancy, fetal distress and normal pregnancies) are provided in Table 3.

It is apparent from Table 3 that the "toxemic" group has the lowest mean of each covariate; the analysis of covariance adjusts for this by increasing the mean value for this group and decreasing the means of the other two groups according to the strength of the relation of each covariate, e.g., maternal age, placental weight, to the variable of response (see also Appendix A and Appendix B).

b(1) Sprout Counts.

The unadjusted and adjusted mean numbers of sprouts for each type of pregnancy are given below:

Means	Normal	Fetal Distress	"Toxemia"
unadjusted	7.87	17.18	22.56
adjusted	7.15	17.17	22.75

The adjusted means were pairwise (normal-fetal distress, normal-"toxemia", fetal distress-"toxemia") significantly

different at $\alpha = .005$.

b(1)i. Effects of placental region. When three placental regions (parachorial, intermediate, parabasal) were considered for each case an analysis of the variability within a given group as well as between the three groups yields a test of the region effect that is free from the effects of the type of pregnancy (and vice versa). In this analysis (Appendix B) we can also test for the type of pregnancy by region interaction (i.e., a lack of constancy of the effect of the type of pregnancy over the different regions).

The mean sprout counts at the three placental levels were different ($P<.001$); each pair was significantly different at $\alpha = .001$.

The region mean counts are given below:

Parabasal	Intermediate	Parachorial
12.75	15.90	18.96

The interaction between the type of pregnancy and the sprout counts when analysed over the different regions was significant ($P<.001$).

All pairs of means within a zone (i.e., normal and "toxemia" in the parabasal zone) or the type of pregnancy (i.e., parabasal and intermediate zone in "toxemia" of pregnancy) were significantly different ($\alpha = .001$). The

characteristics of the mentioned correlations are summarized in Table 4.

b(2). Lumen to Whole Diameter Ratios. The unadjusted and adjusted means of the lumen to whole diameter ratios were not different and therefore only one value is shown below. The means for the different type of pregnancies were pairwise (normal-fetal distress, normal-"toxemia", fetal distress-"toxemia") significantly different at $\alpha = .01$.

Normal	Fetal distress	"Toxemic"
.38	.33	.25

b(2)i. Effects of placental region. The mean lumen to whole artery diameter ratios differed between the three zones ($P<.001$). The smallest ratio was found in the parachorial region and it was .27. The means for the intermediate and parabasal zones were .32 and .37, respectively. All pair of means were significantly different ($\alpha = .001$). The summary effect of the type of pregnancy and placental zones on the lumen to whole artery diameter ratios is illustrated in Table 5.

The overall pregnancy type means were pairwise (normal-fetal distress, normal-"toxemia", fetal distress-"toxemia") significantly different ($\alpha = .01$), as in the analysis of covariance. This holds at $\alpha = .05$ within the parabasal and

intermediate regions, but for the parachorial layer the mean values in the normal and fetal distress groups do not differ. Within the normal and fetal distress groups all pairs of mean values differ ($\alpha = .001$), whereas in the hypertensive group the intermediate and parachorial means show a less significant difference ($\alpha = .05$).

c) The Effect of Maternal Conditions Within the Hypertensive Group. No significant differences in the mean sprout counts or lumen to whole diameter ratios were found among the subgroups pre-eclampsia, chronic hypertension, and renal disease associated with "toxemia" when tested by one way analysis of variance-F ratios. The means and standard deviations ( ) were:

	Pre-eclampsia	Chronic Hypertension	Renal Disease
Sprout Count	22.51 (3.35)	22.18 (3.04)	23.32 (4.40)
Lumen to Whole Diameter Ratio	.26 (.06)	.25 (.09)	.22 (.06)

d) The Relation Between Sprout Counts, Lumen to Whole Diameter Ratios and Other Variables. Pearson correlations between the two variables of response and other variables were examined separately for the three types of pregnancy with the following results.

d(1). Variables recorded for all groups. The variables recorded for all cases were: sprout count (S.C.), lumen to whole diameter ratio (D.R.), maternal age (M.A.), gestational age (G.A.), placental weight (P.W.), birth weight (B.W.), and Apgar score (A.S.). The correlations with a P-value near the $\leq .5(*)$ for each of the three groups and for all cases are shown in Table 6.

The highest correlations for the three groups were: gestational age with birth weight and placental weight with birth weight. These were as expected and showed a tendency for even stronger correlations in the "toxemic" than in the other two groups. Moreover, there is strong evidence for a direct relation between the Apgar score and the gestational age, and the sprout count and the gestational age in the "toxemic" group, whereas no evidence of such relation is apparent in the other two groups. However, one should keep in mind that these correlations could be influenced by the effects of other factors in the "toxemic" group, i.e., by the maternal condition. The inverse relation between the sprout count and lumen to whole diameter ratio means that a larger number of sprouts is accompanied by a greater reduction in the ratio of the lumen to whole artery diameters.

d(2). Variables recorded for the "toxemic" group only. In addition to the variables obtained for all cases, proteinuria (P),

blood pressure (B.P.), and urinary estrogens (U.E.) were recorded for the "toxemic" group. The correlations of the above variables for the "toxemic" group with $P \leq .05$ are summarized in Table 7.

There is evidence of a direct correlation between proteinuria and two of the analysed variables: sprout count and blood pressure. The level of the urinary estrogens is directly related to the gestational age and placental weight and indirectly to the maternal age. All correlations of variables for the "toxemic" group with $P \leq .05$ are represented on scattergrams (Tables 8-14). A scattergram showing the correlation between syncytial sprouts and mean lumen to whole diameter ratios for both the normal and the "toxemic" group is represented in Table 15.

2. Morphological Studies of the Placental Fetal Arteries.

a) The normal fetal stem artery.

a(1). Light microscopic observations. It was difficult to distinguish the arterioles from the venules in the terminal villus, but the larger arteries were readily differentiated from the corresponding veins by the considerable thickness of their muscular coat. In a well preserved placenta, the veins were distended usually with blood, whereas the arteries

were constricted, and in some, the lumen was considerably narrowed. The walls of the arteries showed the conventional three coats, i.e., the intima, media and adventitia (Figs. 10 and 11).

The intima consisted of a single layer of endothelium with prominent nuclei which protruded usually into the lumen. The endothelial layer was supported by a PAS-positive basement membrane. In some instances a slight degree of "hydropic degeneration" of the endothelium was noted. There was no internal elastica lamina separating the intima from the media as observed in other arteries.

The cellular elements of the media were smooth muscle cells; occasionally, they were intermingled with collagenous fibrils. The smooth muscle cells were spindle-shaped and contained central elongated nuclei and cytoplasmic myofilaments which were particularly well demonstrated in sections stained with Masson's trichrome. In the large villous stems and in the large branches, the smooth muscle cells often were disposed longitudinally and circumferentially in bundles, whereas in the medium size branches the smooth muscle cells were still in bundles but tended to run only circumferentially. No elastic tissue was found in the media between these cells.

The adventitia did not form a distinct coat; it was composed of concentric rings of loose fibrous tissue which

merged almost imperceptibly with the surrounding connective tissue stroma. There were also smooth muscle cells in the stroma of the villi; they were separated by varying amounts of connective tissue and were at times "connected" with similar cells lying in the transitional zone between themselves and the media of the blood vessels. Some of these smooth muscle cells could be traced to blood vessel walls but most ended "blindly" in the supporting matrix. These smooth muscle cells were not present in bundles but in discontinuous sheets arranged circumferentially or radially. Most of these cells were spindle-shaped and only occasionally, a "branched" irregular form was intermingled with these. The interstitial smooth muscle cells were most numerous around the stem vessels and diminished gradually toward the periphery of the villi.

a(2). Electron microscopic observations.

By electron microscopy the wall of a smaller caliber stem artery did not appear as compact as by light microscopy. It consisted of a prominent innermost lining composed of endothelial cells whose large portions projected into the lumen "free", i.e., not adhering to the neighbouring cells (Figs. 12-14). Only over a short distance at the basal portion was there an organized apposition between adjacent cells

in the form of a simple junction. Whereas it was not within the scope of the present investigation to study these junctions in detail, it appeared on the basis of the medium power micrographs that they had the general structure as described by Hüttner et al. (1973a; b) for larger arteries. The nucleus was large and often simply folded or bean-shaped (Fig. 14), its nucleolus when present, prominent (Fig. 15) and the chromatin had a distribution characteristic for endothelial cells.

The cytoplasm of the cell that projected into the lumen appeared to have a simpler structure than that at the base. The latter often contained cytoplasmic fibrils (Fig. 14) and a prominent Golgi (Fig. 14) zone whereas in the luminal part there were a few cytoplasmic organelles only. In keeping with other endothelial cells there were only a few small profiles of rough endoplasmic reticulum, a few round mitochondria and numerous caveolae cellulares, particularly at the basal and luminal aspect of the cell. Microvillous projections into the lumen were observed (Fig. 14), but were not a prominent feature.

In most of the cells there were cytoplasmic osmiophilic structures suggestive of the Weibel-Palade bodies (Weibel and Palade, 1964), i.e., the supposed hallmarks of the endothelial cells. However, the tubulo-fibrillar structure of these was not apparent in the cells studied.

The endothelial cells were seen in mitosis on occasion (Fig. 16). It was of interest to note that the junctions of a cell in this phase with the adjacent cells were of a considerable length (Fig. 16).

On the basal aspect the endothelial cells possessed infoldings and extending processes, and rested on a prominent basement membrane. This basement membrane (BM) varied little in thickness, but showed small areas of reduplication or aggregation of its substance.

Adjacent to the medial aspect of the BM were the smooth muscle cells (SMC's) of the medial coat and only very occasionally a few collagen fibrils intervened between them (Fig. 16). The medial SMC's often were of the complex branching variety and surrounded by their own BM. The innermost first "row" was usually circumferential, whereas circular, spiral and longitudinal arrangement of the SMC's was observed in the deeper layers of the media (Figs. 12, 13 and 15). The latter may be construed as representing an overall spiral arrangement of the medial cells (other than those of the innermost layer). In many areas the SMC's of the medial coat immediately adjacent to the endothelium were in close contact by means of cellular processes with the endothelial cells (myoendothelial "junctions"); in these areas there was no basement membrane intervening between the two cell types (Figs. 14 and 15), but there was also no specially organized junction. Of interest also was that the cellular processes of the SMC's in contact with the endothelial cells

appeared slightly but distinctly swollen (Figs. 13, 17 and 18).

The innermost medial SMC's appeared to form a "tight" layer, being in close contact with each other. The remaining medial cells were almost always separated from each other by an interstitial space that increased towards the outer media. The intervening interstitium contained collagen fibrils which increased in number towards the adventitia, and a BM-substance that was either focally accumulated or duplicated over a considerable distance (Fig. 19). Moreover, numerous small cell processes were present, whose nature was not immediately evident (Figs. 12-18). Since these small cellular processes seldom contained cytoplasmic organelles and were not surrounded by a basement membrane, it appeared at first that these belonged to fibroblasts. However, in none of the photomicrographs studied could fibroblasts be identified within the confines of the arterial wall. In fortuitous sections, however, it was possible to establish that processes, morphologically identical with those "free" in the interstitium, were extending from the main body of the SMC's (Figs. 20 and 21). In such instances the processes "penetrated" the surrounding BM of the SMC's and extended "naked" beyond it into the interstitium. These still attached processes appeared slightly swollen and in this respect resembled similar (but much fewer) processes originating from the innermost row of the SMC's and contacting the endothelial cells at the myo-endothelial "junctions" (see above).

All medial SMC's were characterized by the typical fence-like contour and chromatin distribution of their nuclei, cytoplasmic myofilaments, oblong and triangular densities, and numerous caveolae cellulares; only a few mitochondria and a few profiles of rough surface endoplasmic reticulum were usually present. Thorough search failed to disclose the presence of any, even the smallest, elements of elastic tissue in the media.

The transition zone between the media and the surrounding connective tissue was not studied in detail in view of the interest directed in the present study towards the innermost components of the arterial wall.

b) Fetal Stem Arteries in Hypertensive Disorders of Pregnancy. In studying the fetal stem arteries of the placenta it is important to bear in mind that some features of those arteries, e.g. the seemingly thickened wall, may be interpreted as a result of post-mortem changes of the muscular coat; this may result in what appears to be a considerable narrowing of the lumen. Similarly, the contraction of the arteries or tangential sectioning of tissue could alter the shape of the endothelial cells which may appear swollen and bulging, or at times even columnar in shape. Thus all morphological observations have to be interpreted with caution.

b(1) Light microscopic observations. In the affected fetal arteries from hypertensive pregnancies most of the histo-

logical changes occurred in the intimal and medial layers. On the basis of these vascular alterations it appeared possible to consider at least two different types of changes in the arteries involved.

In the first group, represented by changes in arteries of approximately 300 μ of diameter, the intima was characterized by various stages of proliferation of its components and a considerable prominence of the endothelial cells; both processes resulted in a narrowing of the lumen (Fig. 22). The early stage of the intimal change appeared to consist of swelling and irregular proliferation of the endothelial cells; these cells either formed localized "bulgings" which encroached upon the lumen, or affected the entire circumference. The proliferating endothelial cells often formed "bridges" across the lumen (Fig. 23). The presence of cells growing apparently from the subendothelial layer and forming a concentric rim around the lumen was also noted (Fig. 22). This may be interpreted either as a cellular intimal proliferation or, alternatively, as a proliferation of medial smooth muscle cells which assumed a longitudinal direction. In view of the absence of an internal elastic lamina and thus, lack of clear division in these arteries between the media and the intima, the interpretation of these changes may be at best arbitrary by light microscopy.

The media was characterized by the proliferation of the smooth muscle cells and collagen fibers. The smooth muscle

cells were separated from each other by wide intercellular spaces containing a coarse mature collagen; in most of the vessels the smooth muscle cells were circumferentially oriented and had an orderly alignment (Fig. 24), whereas in others their arrangement appeared to be somewhat haphazard (Figs. 25-27). In some cases the morphological features of the smooth muscle cells were not only unusual but considerably more variable and complex (Fig. 28) than those of the spindle form of the smooth muscle cells, i.e., the common cells in the fetal stem arteries. In the arteries with a fully developed lesion the media showed vacuolated smooth muscle cells and there appeared to be also vacuolated extracellular areas considered to contain edema fluid (Fig. 29). It was difficult to determine by light microscopy at all times whether this fluid was intracellular, extracellular or both. The latter features were often accompanied by distortion and fraying of the medial connective tissue (Fig. 29).

The second type of change involved arteries of smaller size (100-150 μ in diameter); these were completely or almost completely obliterated by the proliferation of medial smooth muscle cells, without much participation of the endothelial cells. In this group of arteries the smooth muscle cells in the media were arranged in a concentric laminar fashion, usually reminiscent of an onion skin type of configuration (Figs. 30 and 31). The cells growing from the subendothelial

layer observed in the larger arteries were also present.

Of particular interest were thrombotic and thromboembolic lesions occasionally present in some arteries and veins in placentas of hypertensive pregnancies. The thrombi were usually deposited in one of the major branches of the stalk of the villus, and showed at times certain degree of organization. In some vessels the thrombotic or thromboembolic lesions were recent and composed of layer fibrin and enmeshed blood cells (Figs. 32 and 33), whereas in others the thrombi showed advanced organization, mass of fibrous tissue, usually causing an eccentric narrowing of the lumen (Fig. 34) and sometimes a complete occlusion (Fig. 35). This was interpreted as resulting from the organization of such thrombi or thromboemboli. In these instances, avascularity and fibrosis of the villi supplied by the vessels were also evident. In a few cases "cushions" of acellular hyaline tissue that formed central masses completely occluded the lumen of a vein (Fig. 36). It is assumed that these represented also old organized and hyalinized occlusive thrombi.

An arteritis characterized by a widespread cellular exudate that involved all three coats was noted on occasion (Fig. 37). This process no doubt could induce thrombosis that would result in the obliteration of the lumen of the arteries involved.

In the villous stroma the connective tissue fibers and the smooth muscle cells were more numerous in the hypertensive than in the control groups. The smooth muscle cells were oriented circumferentially around the vessels and were "connecting" one vessel with another, forming bridges in a sigmoidal fashion (Figs. 38 and 39). This stromal arrangement of the smooth muscle cells was not observed in the control placentas.

It should be mentioned in closing that in placentas from normal pregnancies and of fetal distress groups a few fetal stem arteries did show some of the pathological changes similar to those described above. However, the incidence of arteries affected in the placentas of both control groups was low and the degree of involvement of the arteries was very slight as compared with those in placentas of the hypertensive group (see Statistical Analysis).

b(2). Electron microscopic observations. As specified previously, only the fetal stem arteries ranging between 100-300 micra in diameter were examined; these corresponded to the arteries of the same order from normal placentas that were studied by electron microscopy (see above).

The changes observed largely confirmed those seen by light microscopy with respect to several features. Thus, the lumen was either extremely narrowed (Fig. 40) or totally obliterated (Figs. 41-43) and the thickness of the wall was

increased. Several processes accounted for the latter phenomenon. There was an absolute increase in the number of arterial smooth muscle cells (SMC's), many of which now assumed a distinctively circular arrangement (Figs. 40 and 42). Nevertheless, in certain segments of the outer media there was "crowding" of longitudinal SMC's (Fig. 41). In addition, there was a definite increase of collagen fibrils in all interstitial spaces with the notable exception of the space between the innermost layer of the SMC's and the endothelium (Fig. 44). Moreover, there was a considerable proliferation of a basement membrane (BM)-like substance in all spaces, particularly evident between the first and second innermost layers of the SMC's (Fig. 45). In fact, the remarkably intense proliferation of the BM-like substance often created the impression, as if an attempt were made by the second layer of the SMC's to assume the function of the innermost layer of the normal arteries. This was particularly tempting to postulate in view of the fact that the SMC's of the innermost layer often were oriented tangentially or longitudinally rather than circularly as seen in normal arteries (Figs. 40 and 41; Fig. 46). Finally, proliferation of the innermost cells contributed in large measure to the thickening of the wall (Figs. 40-46). Most of these cells proliferating towards and impinging upon the lumen had unmistakable features of endothelial cells (described in the preceding section on normal arteries). Whereas

these proliferating cells could be observed at times "piling up", their nature was clearly evident not only by virtue of their cellular features, but also by the unequivocal cellular junctions between them (Figs. 47 and 48). Helpful in the identification of the endothelial cells were also the Weibel-Palade bodies when present and despite the fact that their tubulofibrillar structure was usually not resolved.

However, at times, some of the proliferating cells had certain features including those of oblong (myofilamentous) densities (Fig. 49) reminiscent of young SMC's rather than endothelial cells. Often, even cells in mitosis (Figs. 40 and 50) with features reminiscent of, or even identical with, those of mitotic endothelial cells (compare with Fig. 34) were noted. These cells could not be identified with certainty as endothelial cells, when there were no cellular junctional complexes present (Figs. 40 and 50). Many subendothelial SMC's often showed the presence of a centriolar complex (Fig. 50).

Electron microscopy was of considerable help in the identification of the vacuolar nature observed by light microscopy within the arterial wall. These vacuoles proved to be intracytoplasmic in nature (Figs. 48 and 51), and involved invariably the processes of SMC's observed also in normal arterial wall. In the luminal area these edematous SMC's processes (Fig. 48) involved those that "participated" in the myoendothelial "junctions", whereas in the media the

edema affected the slender SMC's extensions (Fig. 51). Presumably, when the involvement of either of the two groups of the SMC's = processes reached a "saturation" by an edema fluid, the latter could "spill" over into the interstitium. On occasion, a portion of a membrane bounding a vacuole was, indeed, missing. This could, however, represent an artifact rather than a true discontinuity of a plasma (SMC) membrane (Fig. 51).

IV. DISCUSSION

The results of the present study confirm previous observations (Alvarez et al., 1967; 1969; 1970) that the syncytial sprouts are more numerous in the placentas in hypertensive disorders than in those of normal pregnancies.

The results obtained from the fetal distress group that served as one of the controls showed certain characteristics that warrant a separate analysis.

For the convenience of the discussion the syncytial sprout proliferation in normal placenta will be considered first, followed by the analysis of the same phenomenon in the hypertensive diseases of pregnancy and the fetal distress group.

A. SYNCYTIAL SPROUT PROLIFERATION IN NORMAL PLACENTA

In normal placentas, during the first weeks of pregnancy, syncytial sprouts are large and numerous. The sprout to villus index apparently decreases with advancing maturation of pregnancy and at term only a small number of moderately developed sprouts are usually seen.

In the present study, the mean ratio of sprout to villus in normal pregnancy was 7.87% unadjusted and 7.15% when

adjusted to the variables controlled in the statistical design. Our findings are slightly different than those reported by Alvarez et al. (1967). These authors, while studying the number of sprouts in normal placenta by light and phase contrast microscopy, found that the mean ratio of sprout to villus was 3.55% and 5.22% respectively when related to the number of villi (100) per field unit. However, different results were reported by the same group of investigators (Alvarez and Benedetti, 1971) with a mean ratio of sprout to villus of 7.24% in 100 villi of the normal placenta at term.

The results of the present investigation are in agreement with those reported in the literature with respect to the mean sprout count in the three placental regions; the counts were significantly different in each and in increasing order from the parabasal through intermediate to parachorial level (Table 3).

There is good evidence that the placenta has different zones, each with a unique morphology, and specifically adapted to facilitate the interchange between mother and fetus. In mature placentas, the number of syncytial sprouts at various planes of the fetal cotyledon increased from the parabasal to the other two layers (intermediate and parachorial) (Alvarez et al., 1969; 1970). Moreover, Reynolds et al. (1968) showed that the gas pressure (pO_2 and pCO_2) gradients were higher in, or close by the central zone of the cotyledon, and diminished radially from this zone towards

the subchorial lake. On the basis of these results and their own experience, Alvarez et al. (1970, 1972), and Alvarez and Benedetti (1971) suggested that the different morphological sectors of the placenta were regulated by the regional differences in oxygen tension of the blood in the intervillous space and that the gradient in the number of syncytial sprouts was inversely related to the pO_2 gradient. Hence, it appears that the pattern of circulatory dynamics suggested by Ramsey (1959, 1962), and Ramsey et al. (1963) is responsible for the existence of zones with different oxygen tensions; in turn, the characteristic of the sprout proliferation are determined by the above circulatory dynamics.

B. SYNCYTIAL SPROUT PROLIFERATION IN HYPERTENSIVE DISEASES OF PREGNANCY

The different terminology used by various authors to define the several types of syncytial proliferation made the attempts at comparing the results difficult.

In the present study, the mean ratio of syncytial sprouts to villus was increased in the hypertensive disorders of pregnancy when compared with both control groups. Moreover, the morphologic variations between different areas of the placenta were also evident (Table 4). Similar results have been reported by several authors (Alvarez et al., 1967; 1969; 1970; 1972; Alvarez and Benedetti, 1971; Aladjem, 1968;

Misenhimer, 1972; Cibilis, 1974).

There are indications that a relative hypoxia stimulates the proliferation of syncytiotrophoblast (McKay et al., 1958), and clustering of syncytial nuclei under various conditions of hypoxia was shown to occur in vitro (Tominaga and Page, 1966). It is accepted presently that the syncytiotrophoblast originates from the cytotrophoblast (Tao and Hertig, 1965; Richart, 1961) and that the latter also proliferates under conditions of hypoxia (Fox, 1964b; 1970) and in placentas from "toxemic" patients (Wigglesworth, 1962; Fox, 1964b).

Chronic hypoxia as a consequence of a reduced maternal blood flow has been frequently related to hypertensive disorders of pregnancy (Browne and Veall, 1953; Dixon et al., 1963). Therefore, it is probable that the proliferation of cyto-, and syncytiotrophoblast in this disease group represents a reaction to hypoxia and not a degenerative or "ageing" process as had been believed for a long time (Tenney, 1936; Wislocki and Dempsey, 1943; Paine, 1957; Shanklin, 1958). Other investigators (Alvarez and Benedetti, 1971) postulated that syncytial proliferation may be under the influence of a "placentotrophic" factor that is derived from the pituitary. However, the experimental evidence in support of this theory has not been provided. There are some indications that suggest that the great majority of patients with hypertension of pregnancy have an elevated

level of the hormone chorionic gonadotrophin (H.C.G.) (Smith and Smith, 1934; 1935; Govan et al., 1951; Samaan et al., 1969). Since H.C.G. is synthesized and released by the syncytial layer of the trophoblast one is tempted to postulate that the "repair activity" represented by the trophoblastic proliferation is remarkably successful and the placenta is probably functioning as efficiently as the diminished maternal blood flow allows.

The data obtained in the present study suggests a relation between the syncytial sprout proliferation and the increased gestational age, higher levels of proteinuria and higher Apgar score (Table 6). The same correlation between the number of syncytial sprouts and increased gestational age was noted by Alvarez et al. (1967) who found that in "toxemia" syncytial proliferation reaches maximum values at term and post-mature pregnancies. An unexpected trend was discovered in the present investigation on analysing the Apgar score and the syncytial proliferation in the "toxemic" group as both were positively interacted. Misenhimer (1972) reported that syncytial hyperplasia in hypertensive diseases of pregnancy was closely related to a low one-minute Apgar score in the infants. On the other hand, Garoff and Seppalä (1976) showed that only 11% of infants (25 in 224 cases) in the "toxemic" group had low Apgar scores.

Apparently, the infant exposed to the chronic distress (i.e., "toxemia" of pregnancy) is able to compensate for the

reduced 0_2 supply through circulatory adjustments which include the reduction of the blood supply to non-vital structures and organs such as muscle, skin and lungs via vasoconstriction (O'Sullivan, 1976). Remarkably, the placenta may withstand the loss of almost a third of its functional parenchyma without a resultant fetal embarassment (Fox, 1976); and the trophoblast has the capability to repair itself under conditions of ischemia. Therefore, it seems highly probable that any fetal deprivation that does occur in association with underperfusion of the placenta is not the result of a trophoblastic inadequacy. Finally, there appears to be a direct relation between the sprouts' proliferation and proteinuria (Table 6). This finding is somewhat in agreement with that of Fox (1964,b) who found significant correlation between cytotrophoblastic proliferation and albuminuria. However, one has to consider that this positive correlation may be due to the effect of the type of pregnancy on both variables rather than a cause and effect relation between proteinuria and syncytial proliferation.

C. SYNCYTIAL SPROUT PROLIFERATION IN ACUTE FETAL DISTRESS

There are a number of complications of pregnancy that determine either the acute or chronic fetal distress by mechanisms not entirely understood. When the physiological

state encompassing the maternal-fetal-placental unit is affected over a prolonged period of time ("toxemia", diabetes mellitus) such distress is most probably chronic. On the other hand, when signs of intrauterine anoxia appear just before the delivery or during the labor without satisfactory obstetrical cause, the fetal distress is usually acute. In most cases of acute fetal distress, the fault lies in factors other than the placenta, which, having a considerable reserve capacity, is usually functioning at optimal efficiency.

However, there are instances in which abnormalities of the nutritive and/or respiratory function of the placenta appear to play an important role in the cause of fetal distress (O'Sullivan, 1976). Measurements of placental surface carried out by Clavero and Botella Llusia (1963) in cases of acute fetal distress showed in the majority of these a decrease of the placental area. The same authors (Clavero et al., 1973), studying a similar group later, noted a decreased intervillous blood flow and described the corresponding placentas as "senescent", "hypermature", with accentuated "villous ageing".

Fox (1964b; 1965) found no relation between neonatal asphyxia, fetal distress and syncytial knots. However, he pointed out that there is a positive correlation between the above mentioned groups and increasingly high cytotrophoblastic cell counts.

From the present study it appears that the mean number of sprouts in acute fetal distress is significantly greater than the mean value in the normal group. The morphological differences between the three layers of the placenta observed in the normal and "toxemic" groups were also evident in the fetal distress group (Table 4).

As was discussed above, the magnitude of the trophoblastic proliferation is apparently related to its surrounding pO_2. When chronic fetal distress is caused by a reduced maternal blood flow, a feature believed to be operational in hypertension of pregnancy, there is a proliferation of syncytial sprouts as a consequence of hypoxia (Alvarez and Benedetti, 1971). The clinical manifestations of acute hypoxia also may reflect changes in the utero-placental or placental blood flow (Stembera et al., 1968; Gruenwald, 1974; Oh et al., 1975; O'Sullivan, 1976). A sudden fall in the former, as it may occur in maternal shock, uterine hypertonia, pelvic contractures, or partial detachments of the placenta, results in a fall in blood and tissue pO_2 (Clavero and Botella Llusia, 1963; Berg et al., 1973). Therefore, it is likely that the basic change determining a high number of villous syncytial sprout formation in the acute fetal distress group is a reduced fetal villous blood circulation followed by a placental hypoxia.

Other authors (Alvarez and Benedetti, 197; Cibilis, 1974) suggested that the excessive trophoblastic proliferation develops after certain time and may be absent in cases

of short term "toxemias". However, Tominaga and Page (1966) have demonstrated experimentally that striking changes are present in the syncytiotrophoblast as early as 6 hours after subjecting it to a hypoxic environment. If then, in the placenta from acute fetal distress, proliferation of the syncytial sprouts represents a response to ischemia, is the degree of proliferation a guide to the degree and duration of that ischemia?

It is very difficult to determine with accuracy when the development of acute fetal distress begins, particularly in the absence of an apparent cause other than the ill-defined "placental insufficiency". Moreover, this is even more difficult when a fetus with an undetected chronic distress manifests it only when exposed to the additional stress during the labour.

It cannot be claimed that by determining the extent of syncytial proliferation one is establishing the morphological expression of the functional status of the placenta. It is probable nevertheless, that the assessment of the degree of this proliferation may prove to be a helpful method for the evaluation of the acute placental hypoxia.

D. CHANGES IN THE FETAL STEM ARTERIES IN HYPERTENSIVE DISEASES OF PREGNANCY

The importance of a correct interpretation of the changes in the fetal vessels of the mature placenta cannot be over-estimated. The assessment of the normal arterial structure is difficult and the morphological changes brought about by the process of "ageing" of the placenta should be taken into consideration when evaluating the status of the arterial structure. It was for that reason that along with the morphometric study, a morphological evaluation of these arteries in normal pregnancies was undertaken in order to allow for a comparison with the status of similar arteries in the hypertensive diseases of pregnancy.

Several investigators (Riviere, 1930; Patterson et al., 1938; Hunt et al., 1940) have reported on the presence of "obliterative endarteritis" in placentas from the hypertensive group, but the first systematic analysis of the microscopic features of the placental fetal vessels in "toxemia" of pregnancy should be credited to Paine (1957) and Fox (1967). They characterized the lesions in the arteries as an endothelial proliferation and medial thickening. More recently, Bender et al. (1976) reported similar changes in various complications of pregnancy, including those of hypertension of pregnancy. For convenience of the discussion, the intimal changes will be analysed first,

followed by a consideration of the medial alterations. Exception will be made when discussing the changes common to both, intima and media.

In the present study diffuse swelling and haphazard proliferation of the lining endothelial cells was a feature consistently found in the affected areas of the hypertensive group (Figs. 22 and 23). Placentas from the fetal distress group showed similar changes, but significantly fewer arteries were affected. The extent of the intimal lesion was considerable and best demonstrated in serial sections in which the arteries were narrowed or obliterated over long segments. The present findings are similar to those observed by other investigators. Thus, thickening of the intima in stem fetal arteries of the placenta was first recognized by Ackermann (1891) and further stressed by several authors (Eden, 1897; Hunt et al., 1940; Burstein et al., 1963; Fox, 1967). Ackermann (1891) believed that the reduction of pressure in the vascular system of the placenta was responsible for the narrowing of the arteries. Eden (1897) suggested that the process might be part of the "natural forces of evolution and decay", whereas Hunt et al. (1940) stated that the endothelial changes were induced by fetal hypercholesterolemia. The possibility of endothelial proliferation as part of an immunological reacion on the vascular wall was considered by Burstein et al. (1963), and Fox (1967). Moreover, Fox suggested that the fetal

hemodynamic changes occurring in response to placental ischemia could be involved also in the changes affecting the endothelium. Similarly, Bender et al. (1976) postulated that the anoxia, secondary to the reduction in the placental blood flow, causes the endothelial proliferation. According to them, an increased CO_2 saturation leads to loosening and swelling of the endothelium followed by the intimal proliferation.

In the present study, the deposition of an amorphous material was noted in some of the arteries affected, in addition to the proliferative reaction of the endothelium. Similar findings were reported by several authors (Gruenwald, 1961; Burstein and Blumentahl, 1962; Blumenthal et al., 1960; Löhr et al., 1972) and interpreted as being possibly of immunologic origin (Blumenthal et al., 1960), or the result of the deposition of glycoproteins (Burstein and Blumenthal, 1962). By electron microscopy this material was similar to that of the basement membrane (Figs. 43 and 45).

Whereas electron microscopic examination largely confirmed the light microscopic observations, it provided in addition a new information of considerable interest. Thus, it became apparent that the vacuoles evident by light microscopy in the wall of a stem artery from placenta in "toxemia" do not represent fat droplets extracted in the process of tissue preparation, but are edematous cellular processes (Figs. 41, 45, 48, and 51).

Numerous slender cellular processes were "discovered" on electron microscopic examination to be present in the interstitium of normal arterial media. These were observed to originate from the medial smooth muscle cells from whose "bodies" they extended into the intercellular space "naked", i.e., without carrying the basal membrane of the cells (Figs. 20 and 21). In "toxemia" these processes become distended with edema fluid imparting the vacuolar appearance upon the media (Figs. 48 and 51). Similarly, those smooth muscle cell extensions that make contacts with the endothelial cells at the myoendothelial junctions also become edematous (Figs. 41, 48 and 49) giving rise to the vacuoles in the endothelial region of the artery. These processes, too, are devoid of the basal lamina of the smooth muscle cells. It is tempting to postulate that the cellular basal lamina usually protects the cell from the intracellular edema. Alternatively, one could postulate that the smooth muscle cells that are devoided of the basement membrane and either extend to make contacts with the endothelial cells or are free in the interstitium are specialized cellular components; these are capable of absorbing edema fluid that otherwise, if it remained extracellular would damage the extracellular components of the arterial wall. To the best of the author's knowledge, the above observations were not reported in the literature to date.

The present study indicated that the media of the

compromised arteries of hypertensive diseases of pregnancy almost always was thickened by proliferating smooth muscle cells and fibrous tissue (Figs. 24-27). Moreover, in some arteries from the hypertensive group there were "cushions" of acellular hyaline tissue, probably representing old organized and hyalinized occlusive thrombi (Fig. 34 and 35).

Similar vascular changes, i.e., proliferation of the smooth muscle cells, were occasionally found in some placentas from the fetal distress group, although these were considerably less severe.

What initiates the process whereby the obliteration occurs is unknown, but almost certainly a variety of stimuli may be involved. One may offer only some thoughts and speculations on these matters having no evidence other than morphological to support them. It would appear from the present findings that the endothelial proliferation and the thickening and obliteration of the fetal arteries are different stages of the same process. Moreover, in accord with Bender et al. (1976) it is believed that the vascular obliteration of the placental arteries should not be considered as a post-mortem alteration.

With the above considerations in mind, the theories that have been proposed to explain the development of the so-called "obliterative endarteritis" of the fetal villous arteries will be discussed.

E. THEORIES ON PATHOGENESIS OF OBLITERATION OF FETAL STEM ARTERIES

The possible role of placental ischemia in the etiology of "toxemia" of pregnancy has attracted attention for many years. Several authors (Page, 1939; Beker, 1948; Bastaanse and Mastboom, 1949; 1950) have suggested that a diminution of the blood supply to the maternal circulation of the placenta could evoke a rise of blood pressure in the mother. Subsequent estimates of uterine blood flow in normal pregnancy and in pre-eclampsia (Browne and Veall, 1953; Assali et al., 1954; Morris et al., 1956; Moore and Myerscough, 1957; Taylor et al., 1958; Landesman and Knapp, 1960; Smith, 1970) indicated that in the presence of hypertension the rate of maternal blood flow through the placenta was signficantly reduced. Whether such changes result only from an alteration in the utero-placental vasculature or whether the fetal vessels of the placenta also play a role has not been established.

The tonus of the fetal placental vessels appears to be dependent upon the 0_2-tension of the circulating blood. In isolated preparations of fetal placental vessels vasoconstriction may be induced by high oxygen tension, while vasodilatation occurs on exposure to high CO_2 (Schmitt, 1922; Ueda, 1931; Myberg and Westin, 1957). In contrast, fetal vessels in vivo show less local effect from changes in the tension of this blood gas. Nevertheless, severe asphyxia

may cause vasoconstriction, presumably because of the release of catecholamines from the fetal adrenals (Walsh et al., 1974). Several investigators (Paine, 1967; Fox, 1967; 1969; Bender et al., 1976) have explained the obliterative endarteritis as a consequence of placental ischemia and low saturation of oxygen of the fetal blood during gestation. Whereas the action of various stimuli may trigger the obliteration of fetal arteries, it is possible that in some patients hypoxia may play the most important role in the mechanism of this vascular alteration. This view is strengthened by the discovery of Thompson and Tickner (1949) that the placental mono-amine oxidase, whose specific action is to destroy vaso-constrictor amines such as adrenaline, is inactivated by a decreased oxygen tension and, therefore, presumably by placental ischemia. It remains to be established whether this or another, yet unknown, enzyme indeed plays a role in the above reactions.

In contrast to arteries at other sites, placental vessels have a relatively thick muscular coat. The present study showed that in the affected arteries the media is considerably thickened by proliferating smooth muscle cells (S.M.C.). Factors that may induce S.M.C. proliferation and medial hypertrophy are not all known, but one of these is almost certainly a prolonged vasoconstriction or an increase of vascular tone (Wagenwoort, Wagenwoort, 1970). Whether this is somehow related to the intensity of vaso-

constriction, the frequency of vasoconstrictive "bouts", or other factors, is unknown. It seems likely that, as in the systemic circulation, this proliferation may be stimulated by intense vasospasms. It is also possible that in time this growth of smooth muscle cells further increased the pressures by progressively reducing arterial distensibility, and thereby augmenting the vascular resistence. That could explain the findings of Brinkman et al. (1969) that in hypoxia the placental vascular resistence rose by 11% to 17%.

There are many substances that are thought or known to affect the placental vasculature. Thus, it was shown that 5-hydroxy-tryptamine and histamine cause a pronounced vasoconstriction (Mancini and Gautieri, 1964; Tulenko, 1976), whereas epinephrine, norepinephrine, and l-epinephrine produce a relatively slight vasoconstriction of isolated placental vasculature (Eliasson and Astrom, 1955; Mancini and Gautieri, 1964). Recent evidence indicates that the small blood vessels of the human placenta are very sensitive to the vasoconstricting properties of angiotensin II (Tulenko, 1976) and prostaglandins E_2 (Rankin and Phernetton, 1976).

In normal human pregnancy there is a decreased blood pressure response to pressor materials (Page, 1972), but in hypertensive diseases of pregnancy there is an increased sensitivity to the pressor effects of vasopressin (Dieckmann

and Michel, 1937; Browne, 1946), norepinephrine and to angiotensin II (Chesley et al., 1965; Talledo et al., 1968). Moreover, Rankin and Phernetton (1976) postulated that the administration of prostaglandin E_2 (PGE_2) and oxytocin to the mother is followed by vasoconstriction of the umbilical circulation and fetal membranes. The authors suggested that this could result from the release of PGE_2 by oxytocin from the placental tissue, or from oxytocin and PGE_2 both releasing the same vasoconstricting substances into the fetal circulation.

It is known that larger quantities of sodium are retained in pre-eclampsia than in normal pregnancy (Little, 1965).

Studies with isolated arteries indicate that a raised sodium content increases the reactivity of the vessels (Burks et al., 1971). Therefore, it is probable that the increased responsiveness of the fetal arteries in hypertension of pregnancy may be brought about by increased concentrations of sodium within the arterial wall; the increased sodium content is probably associated with the extracellular mucopolysaccharides of the arterial wall (Harris, 1970). Page (1972) suggested that the sodium retention in the arterial wall may be enhanced by the influence of steroid hormones. Whereas it is possible to speculate on the nature of some of the factors increasing vascular reactivity to different stimuli, the mechanism by which these stimuli interfere with vascular tone remains an enigma.

As postulated by Burstein et al., (1963), and Fox (1967) immunologic processes may play a role in the etiology of some cases of obliterative endarteritis but the nature of this potential relation also is unexplained. There may exist an individual susceptibility or hyperreactivity of the fetal arteries as is suggested by the fact that the obliteration of fetal arteries is not present in all cases exposed to the same stimuli.

Several investigators (Becker, 1963; Koening, 1972; 1973; Bender et al. 1976) suggested that the intimal proliferative processes may cause fetal death. Becker (1963), and Bender et al. (1976) have described the presence of arterial recanalization and suggested that this may be an "attempt at compensation" in cases of threatening or overt placental insufficiency.

In the present study, there was no evidence of actual recanalization of the fetal arteries. At times, the presence of intraluminal thin dividing "walls" simulating intra-arterial septa was brought about by the proliferating endothelium (Fig. 23). As suggested by Theuring (1968) these changes should be considered as a phase of the active obliterative process rather than a recanalization attempt. It is probable that if the obliteration of the fetal vessels takes place in a large area of the placenta this could lead to an acute or chronic insufficiency state. If this hypothesis be true, alterations in the function of the

fetal vasculature may be as critical for normal fetal growth and development as actual deficiencies in the transfer of specific nutrients to the fetus, or deficiencies in the utero-placental circulation.

There are at least three levels at which an increase or decrease of the oxygen transport may be influenced in the human placenta: at the utero-placental circulation, at the umbilical circulation and at the diffusion capacity of the villi. A compromise of each of these parameters could serve as an explanation of the placental insufficiency and account for the obliteration of fetal stem arteries. First of all, the required increase of the umbilical circulation which takes place with fetal age may be prevented in the presence of obliteration of the placental fetal arteries (Walsh et al., 1974). On the other hand, it has been proposed, and experimental data supports this, that villous fibrosis may be a consequence of a reduced fetal villous blood flow in those villi supplied by the obliterated vessels (Fox, 1965; 1969; Myers and Fujikura, 1968). Moreover, as has been demonstrated in pre-eclamptic toxemia (Fox, 1967; Aladjem, 1970), this reduced vascular area may be further compromised by the presence of avascular degeneration of the villus, where capillary area is absent. Therefore, the required increase of the oxygen diffusion in a progressed state of pregnancy would not take place. The reduced villous vascularity may lead to a loss of the

villous "pulse", which apparently plays a role in the intervillous circulation of the normal placenta (Ten Berge, 1960). This may produce stagnation of the maternal blood with subsequent elevation of pressure in the intervillous space and, as a consequence, a reduction in the maternal blood flow (Fox, 1975).

In the present study, thrombi of fetal stem arteries showing advanced organization were more common in hypertensive diseases of pregnancy than in the control groups (Figs. 32-35). There was no evidence forthcoming from the study to indicate the possible causes of fetal arterial thrombosis. However, it is possible that changes of the electrical potential in the already damaged endothelium in the obliterating arteries may provide conditions favourable to platelet aggregation and intravascular coagulation (Srinovasan and Sawyer, 1970).

Thrombosis in fetal stem arteries was found to occur more frequently in placentas from diabetic women (Driscoll, 1965) than in normal and other pregnancies. Gruenwald (1963) showed that placentas from babies who had developed fetal distress in utero for no obvious reason occasionally showed multiple thrombotic occlusion of the fetal stem vessels; Fox (1966) stated that thrombosis of a single fetal artery was without deleterious effect on the well-being of the fetus.

It was observed in the current investigation that the

placentas in the hypertensive group showed a marked proliferation of the connective tissue and smooth muscle cells affecting the stem villi. Changes in the stroma of the villi characterized by excessive deposition of coarse adult type of collagen has been described by Paine (1957), Salvatore (1968) and Dewhurst (1976) and considered by them as an accelerated "ageing" process of the placenta. Similar changes were described by Emmrich (1966), Justus et al. (1968) and Bender (1976) in placentas from stillborn babies. It was suggested by Julian et al. (1971) that a transformation of the smooth muscle cells into fibroblasts may be responsible for the increased collagenization of the villi. Moreover, as stated by Becker (1975) the collagenous stroma could be a true mechanical hindrance to the transformation of the capillaries into sinusoids, thereby compromising the exchange area of the placenta. Another distinctive feature characterizing the hypertensive group in this investigation was the way in which the smooth muscle cells were oriented in the stem villi (Figs. 38 and 39) i.e., forming bridges between the main vessels. There seems to be no plausible explanation for this phenomenon, but it is probable that by imparting a tension upon the outer part of the vessels these cells represent a compensatory mechanism counteracting the vasoconstriction prevailing in "toxemia" of pregnancy.

The comparison of findings in the fetal vessels in the different sub-groups of hypertension of pregnancy showed no

significant differences between the pre-eclamptic group and those with a history of a chronic renal or hypertensive disease This suggests that regardless of the nature of the maternal hypertension the vascular alteration of the placenta that develop show similar characteristics.

The only factor with which the obliteration of the fetal arteries was significantly associated was the number of syncytial sprouts (Table 6). Regarding this point one may presume that the same factors, i.e., a chronic hypoxia affecting the trophoblastic proliferation may also influence directly or indirectly the fetal vasculature of the placenta.

Open for further exploration is the question of what biochemical alterations occur in the uterus and placenta as the result of ischemia and whether specific substances are released in the utero-placental circulation which influence the fetal vessels. In keeping with other data, it is apparent from the present study that the placental fetal vasculature "participates" in the process operating in the hypertensive ("toxemic") diseases of pregnancy, probably by inducing a reduction of effective fetal exchange area.

V. SUMMARY AND CONCLUSIONS

Notwithstanding the marked improvement in the early diagnosis and treatment of "toxemia" of pregnancy this condition remains as one of the major causes of maternal and perinatal morbidity and mortality. This is not surprising in view of the fact that only the symptoms and signs of the disease are being treated while the basic underlying mechanisms remain largely unexplained.

There have been many investigations of placental morphology in "toxemia" of pregnancy. However, studies relating to changes in the fetal circulation in "toxemia" are few and contradictory. The present study was undertaken to assess morphometrically and morphologically the status of the fetal stem arteries of the placenta in hypertensive diseases of pregnancy. The studies were carried out on placentas of fifty hypertensive patients, fifty placentas of normal pregnancies and fifty from a group of acute fetal distress; the two last groups served as controls.

For morphometry of the fetal stem arteries, sixty arteries in each case were evaluated and the diameter of the lumen and the total diameter of the arteries were determined and recorded on devised tables, thus giving an index which expressed the lumen to whole artery ratio.

These measurements were restricted to arteries of the 3rd order which had a calibre from 100 to 300 micra. Several maternal and neonatal clinical variables (maternal age, gestational age, placental weight, infant's weight at birth and the Apgar score at 1 minute and 5 minutes) were considered for all groups and, in addition, some for the hypertensive group alone (proteinuria, blood pressure and the urinary estrogens). Furthermore, the proliferation of the villous syncytiotrophoblastic sprouts was studied separately as a guide to the severity of the placental ischemia, and correlated with the morphometric studies of the fetal arteries and the clinical variables.

The statistical evaluation involved the analyses of covariance and variance. The relation between variables was examined by computing Pearson correlations for all cases and for the hypertensive group alone.

For morphological studies placental fetal arteries from "toxemia" and normal pregnancies were processed for light and electron microscopy.

Morphometrically, there was a significant reduction in the lumen to whole diameter ratios in the toxemic group as compared with both controls. These findings sugg... that in many cases of "toxemia" of pregnancy the inadequate fetal circulation may lead to a reduced fetal blood flow through the placenta, to an elevation of pressure in the intervillous space, and indirectly, to a reduction in the utero-placental blood flow, thus causing an inadequate

fetal oxygenation. The mean lumen to whole diameter ratio also differed between regions of the placenta in all groups, the most marked reduction being in the parachorial region and the least prominent in the parabasal zone.

No significant differences in the mean lumen to whole diameter ratio were found among the three sub-groups of the toxemic pregnancies, i.e., the pre-eclamptic, essential hypertension and renal disease associated with hypertension of pregnancy. The relation between the lumen to whole diameter ratios and the syncytial sprout proliferation showed that an increased reduction of the former was associated with an increase in the number of sprouts. This negative correlation suggested that the same factors influencing the sprout proliferation in "toxemia" of pregnancy, i.e., hypoxia, are also affecting the placental fetal stem arteries. The other variables analyzed in this study did not show a significant correlation with the lumen to whole diameter ratio of the fetal stem arteries.

Changes in arterial architecture were observed by both light and electron microscopy. The morphological changes appeared to affect two different components of the arterial wall, i.e., the endothelium and the media, and the surrounding stroma. The earliest alterations consisted of an endothelial proliferation leading to a narrowing of the lumen. This lesion was conspicuous in the larger vessels but almost absent in the smaller ones. Subsequently, there was

proliferation of subendothelial cells and smooth muscle cells probably derived from the medial layer. Several changes were noted in the media: the proliferation of the smooth muscle cells and fibrous tissue was followed by a vacuolation in the muscular wall and finally, a degeneration of the smooth muscle cells. Other lesions of the fetal stem arteries, i.e., thrombi and arteritis were less commonly observed. In the villous stroma of the "toxemic" placentas the smooth muscle cells tended to form bridges between the fetal arteries. This extravascular arrangement of the smooth muscle cells was not observed in the control placentas.

Whereas the morphological features of the placental trophoblast and those of the fetal stem arteries are not pathognomonic for the hypertensive disorders of pregnancy, the extent of the changes described and the number of both components affected in a given placenta is suggestive of the conditions under consideration.

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TABLE 1

VASCULAR ANATOMY OF THE HUMAN PLACENTA

Summary of the main theories about the fetal vessels distribution in the fetal and maternal surfaces

	Bøe (1951)	Romney and Reid (1951)	Crawford (1956;1962)
CHORIONIC VESSELS		Primary ↓ Secondary ↓ Tertiary ↓	Primary division ↓ Secondary division ↓ Tertiary division ↓ perforating
COTYLEDONARY VESSELS	Truncus Chorii (main stem villus) ↓ Rami Chorii* (villus stem of 2nd order) ↓ Ramuli Chorii (minor villus stem) ↓ Villi Chorii *including anchoring villi	Long vessels Short vessels Vessels of intermediary length	arterial branches Primary trunk ↓ Primary division ↓ Secondary division ↓ Tertiary division

	Arts (1961)	Reynolds (1967)	Krohn et al. (1970)	Sandsted (1974)
CHORIONIC VESSELS	1st order ↓ 2nd order ↓ 3rd order ↓ 4th order ↓ perforating vessels (or villus vessels of 1st order)			Allanto-chorionic arteries ↓ ↓ Disperse Pattern — Magistral Pattern (Shordania, 1929)
COTYLEDONARY VESSELS	Villus vessels of 2nd order ↓ Villus vessels of 3rd order ↓ Preterminal vessels ↓ Terminal vessels	Stem arteries of the central cotyledons 1st order vessels ↓ 2nd order vessels ↓ 3rd order vessels	Main cotyledonary artery ↓ Fetal intracotyledonary artery ↓ ↓ Type A — Type B	Primary cotyledonary artery ↓ Secondary branch ↓ Tertiary branch Non-branching peripheral arteries

TABLE 2

PLACENTAS OF HIGH RISK PREGNANCIES

(500)

1.	"Toxemia" of Pregnancy	138
2.	Fetal Distress	79
3.	Failure to Progress (Prolonged Labour)	48
4.	Premature Labour	32
5.	Premature Rupture of Membranes	30
6.	Hemorrhage in 3rd Trimester	28
7.	Possible Cephalo-Pelvic Disproportion	19
8.	Postmaturity	16
9.	Abruptio Placentae	15
10.	Diabetes Mellitus	13
11.	Cesarean Section (monitored)	11
12.	Repeated Abortions	11
13.	Small for Dates Babies	10
14.	Perinatal Infections	7
15.	Prematurity	7
16.	Rh Immunization	6
17.	Polyhydramnios	5
18.	Twinning	5
19.	"Chronic Placental Insufficiency"	4
20.	Infertility	4
21.	History of Stillbirth	3
22.	Miscellaneous	9
	TOTAL	500

TABLE 3

MEANS OF COVARIATES BY THE TYPE OF PREGNANCY (±S.D.)

COVARIATE	NORMAL	FETAL DISTRESS	TOXEMIC
Maternal Age	25.4 ± 4.5	25.3 ± 3.4	25.1 ± 5.3
Gestational Age	40.0 ± 1.1	39.9 ± 1.5	38.5 ± 2.8
Placental Wt.	455.4 ± 90.4	466.2 ± 81.7	428.5 ±122.5
Birth Wt.	3305.0 ±407.1	3370.1 ±487.4	3061.2 ±847.6
Apgar Score	9.2 ± .5	8.2 ± 1.5	7.9 ± 1.8

TABLE 4

THE EFFECT OF PREGNANCY TYPE AND PLACENTAL REGION ON SPROUT COUNTS

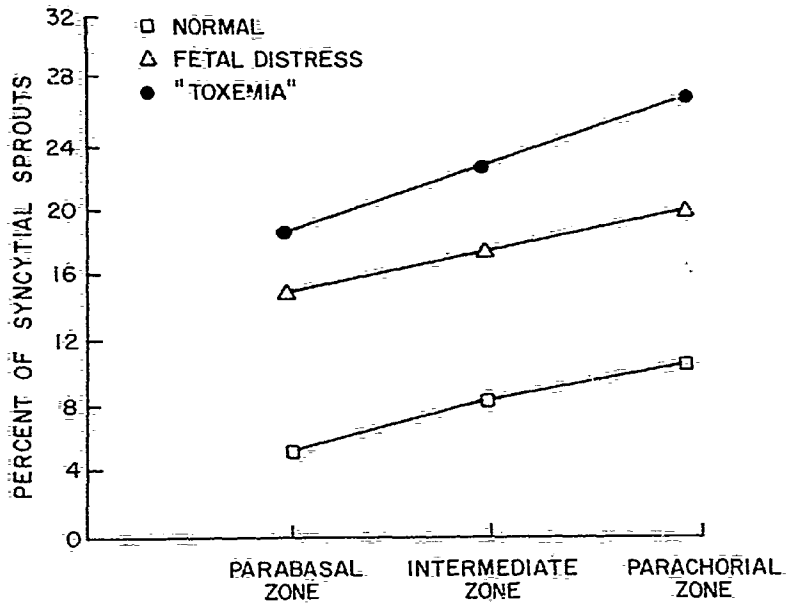


TABLE 5

THE EFFECT OF PREGNANCY TYPE AND PLACENTAL REGION ON LUMEN TO WHOLE ARTERY DIAMETER RATIOS

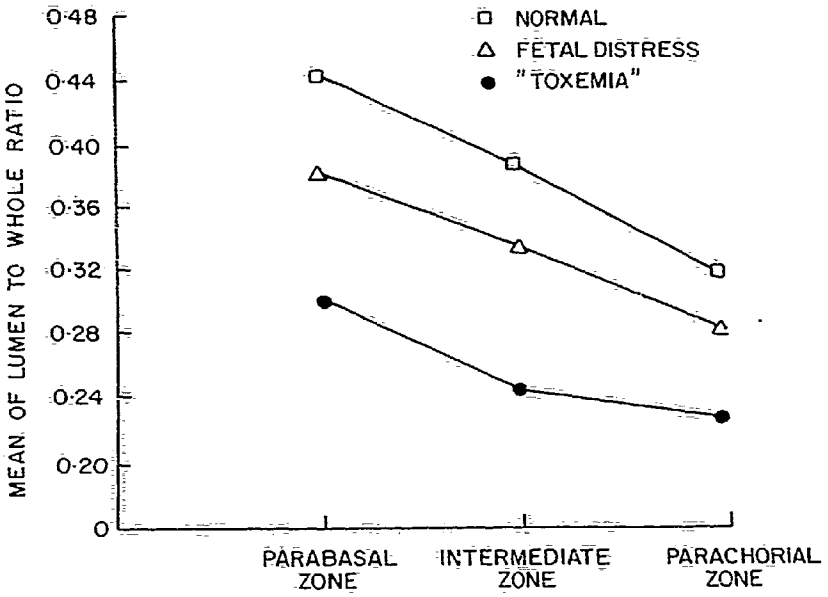


TABLE 6

CORRELATION COEFFICIENTS AND P-VALUES

FOR EACH OF

THREE PREGNANCY TYPES AND FOR ALL CASES

Variables	Normal	Fetal Distress	Toxemic	All Cases
SC, DR	-.30 (.02)*	-.12 (.21)	-.28 (.03)*	-.56 (.001)*
SC, AS	.18 (.10)	-.01 (.33)	.27 (.03)*	-.29 (.001)*
SC, GA	.08 (.29)	.04 (.38)	.26 (.03)*	-.18 (.01) *
SC, MA	-.21 (.07)	.20 (.08)	-.11 (.22)	-.05 (.26)
DR, BW	-.003(.49)	-.41 (.002)*	-.14 (.39)	.03 (.34)
AS, GA	.06 (.34)	.06 (.34)	.59 (.001)*	.44 (.001)*
AS, PW	-.21 (.07)	.18 (.10)	.33 (.01) *	.20 (.008)*
AS, BW	.02 (.45)	-.02 (.46)	.48 (.001)*	.32 (.001)*
MA, GA	.03 (.43)	.14 (.16)	-.20 (.08)	.07 (.20)
MA, PW	.04 (.39)	.03 (.43)	-.23 (.06)	-.09 (.14)
GA, PW	.03 (.41)	.20 (.09)	.51 (.001)*	.37 (.001)*
GA, BW	.35 (.007)*	.55 (.001)*	.74 (.001)*	.70 (.001)*
PW, BW	.67 (.001)*	.67 (.001)*	.71 (.001)*	.68 (.001)*

* $P \leq .05$

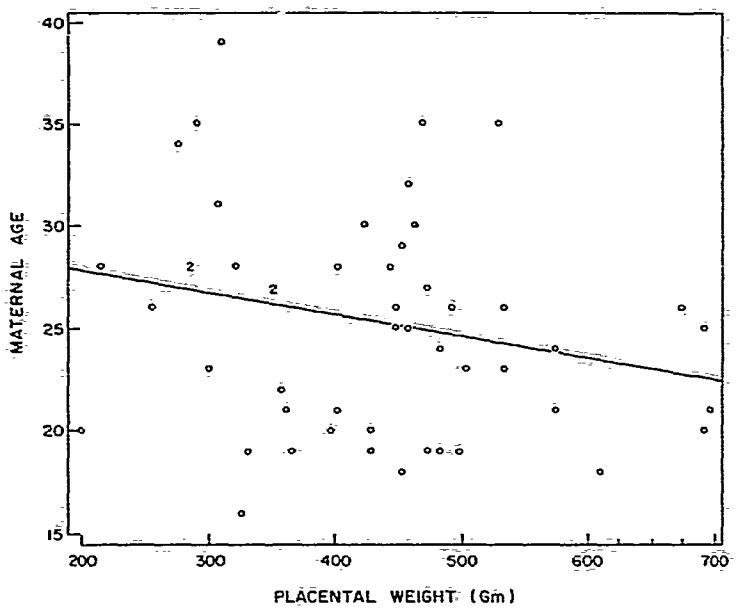
TABLE 7

CORRELATION COEFFICIENTS AND P-VALUES FOR VARIABLES MEASURED ONLY FOR "TOXEMIC" PREGNANCIES

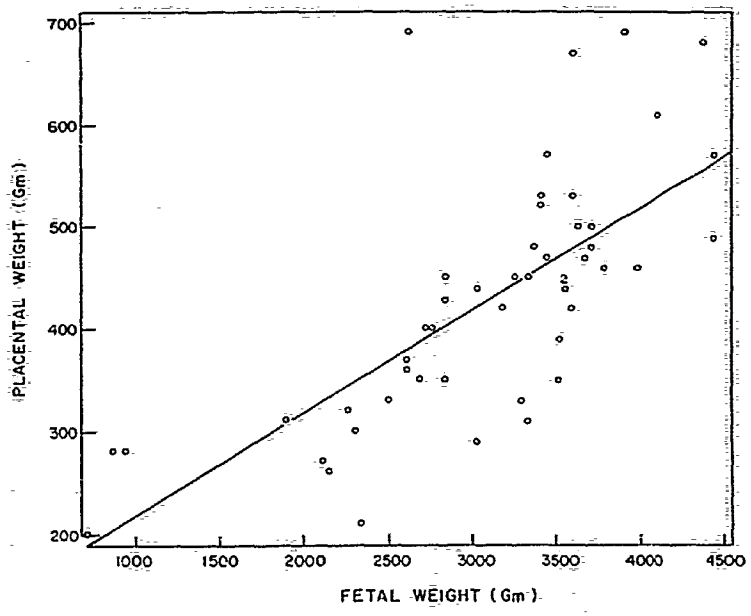
Variables	r	P	n
SC, P	.35	.01	50
MA, UE	-.51	.02	16
GA, UE	.44	.05	16
PW, UE	.68	.002	16
P, BP	.45	.001	50

Table 8: Scattergram demonstrating the relation between the placental weight (Gm) and the maternal age in the "toxemic" group. Multiple observations are identified by a number.

Table 9: Scattergram demonstrating the relation between the placental weight (Gm) and the fetal weight (Gm) in the "toxemic" group. Multiple observations are identified by a number.


MATERNAL AGE
40
35
30
25
20
15
200
300
400
500
600
700
PLACENTAL WEIGHT (Gm)

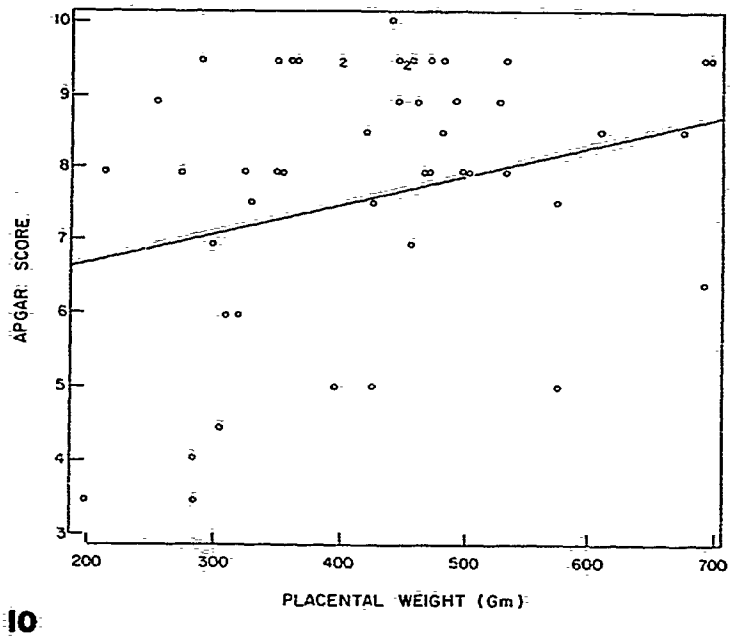
8


PLACENTAL WEIGHT (Gm)
700
600
500
400
300
200
1000
1500
2000
2500
3000
3500
4000
4500
FETAL WEIGHT (Gm)

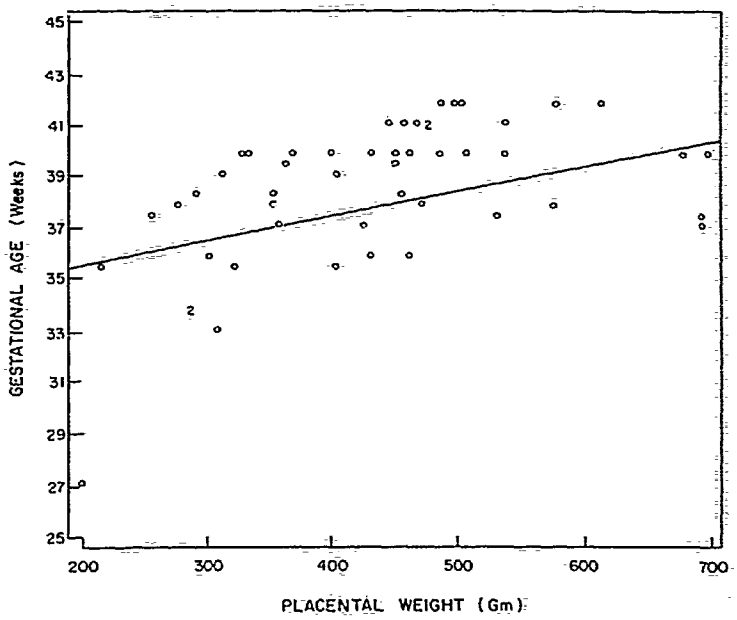
9

Table 10: Scattergram demonstrating the relation between the placental weight (Gm) and the Apgar score in the "toxemic" group. Multiple observations are identified by a number.

Table 11: Scattergram demonstrating the relation between the placental weight (Gm) and the gestational age (weeks) in the "toxemic" group. Multiple observations are identified by a number.


APGAR SCORE
10
9
8
7
6
5
4
3
200
300
400
500
600
700
PLACENTAL WEIGHT (Gm)

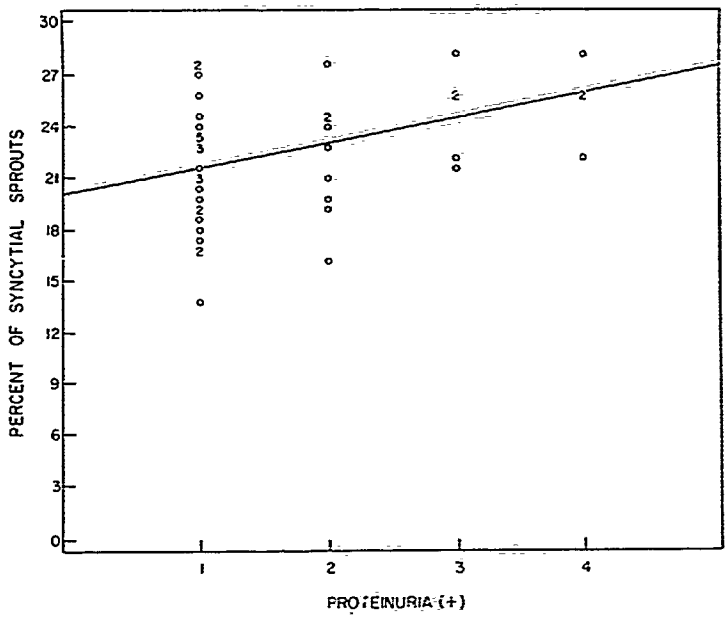
10


GESTATIONAL AGE (Weeks)
45
43
41
39
37
35
33
31
29
27
25
200
300
400
500
600
700
PLACENTAL WEIGHT (Gm)

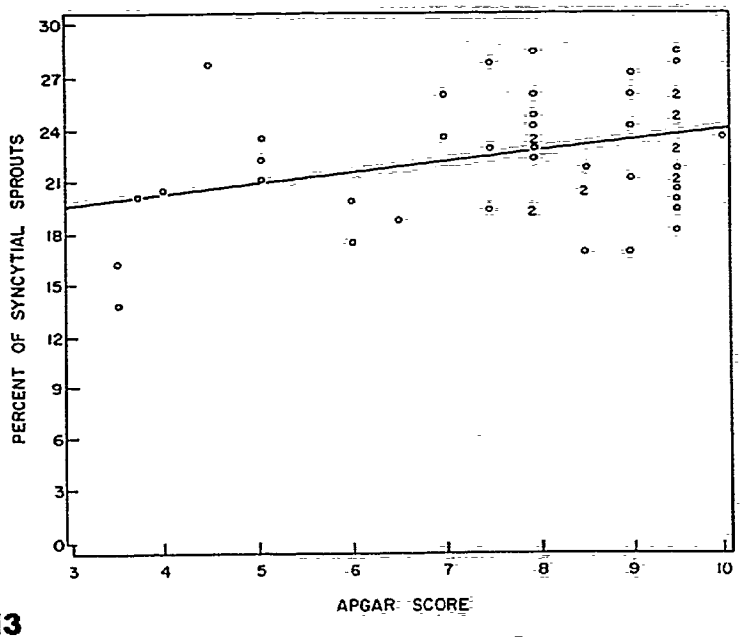
11

Table 12: Scattergram demonstrating the relation between proteinuria (+) and percent of syncytial sprouts in the "toxemic" group. Multiple observations are identified by a number.

Table 13: Scattergram demonstrating the relation between Apgar score and percent of syncytial sprouts in the "toxemic" group. Multiple observations are identified by a number.


PERCENT OF SYNCYTIAL SPROUTS
30
27
24
21
18
15
12
9
6
3
0
1
2
3
4
PROTEINURIA (+)

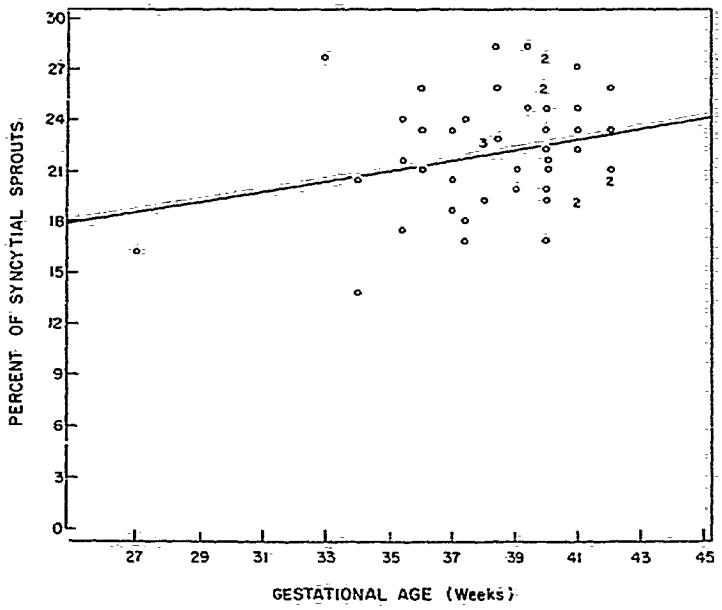
12


PERCENT OF SYNCYTIAL SPROUTS
30
27
24
21
18
15
12
9
6
3
0
3
4
5
6
7
8
9
10
APGAR SCORE

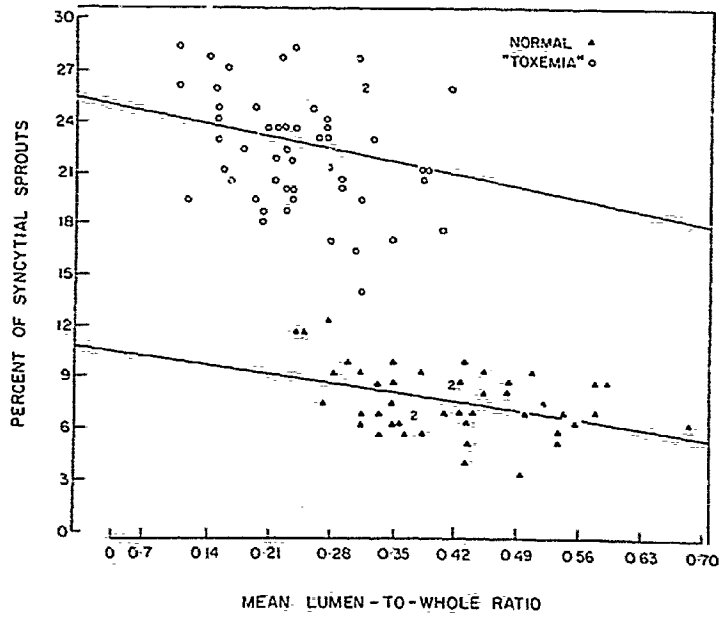
13

Table 14: Scattergram demonstrating the relation between gestational age (weeks) and percent of syncytial sprouts in the "toxemic" group. Multiple observations are identified by a number.

Table 15: Scattergram demonstrating the relation between the means lumen to whole diameter ratios of the fetal arteries and percent of syncytial sprouts in "toxemic" and normal groups. Multiple observations are identified by a number.


PERCENT OF SYNCYTIAL SPROUTS
30
27
24
21
18
15
12
9
6
3
0
27
29
31
33
35
37
39
41
43
45
GESTATIONAL AGE (Weeks)

14


NORMAL ▲
"TOXEMIA" o
PERCENT OF SYNCYTIAL SPROUTS
30
27
24
21
18
15
12
9
6
3
0
0
0·7
0·14
0·21
0·28
0·35
0·42
0·49
0·56
0·63
0·70
MEAN LUMEN-TO-WHOLE RATIO

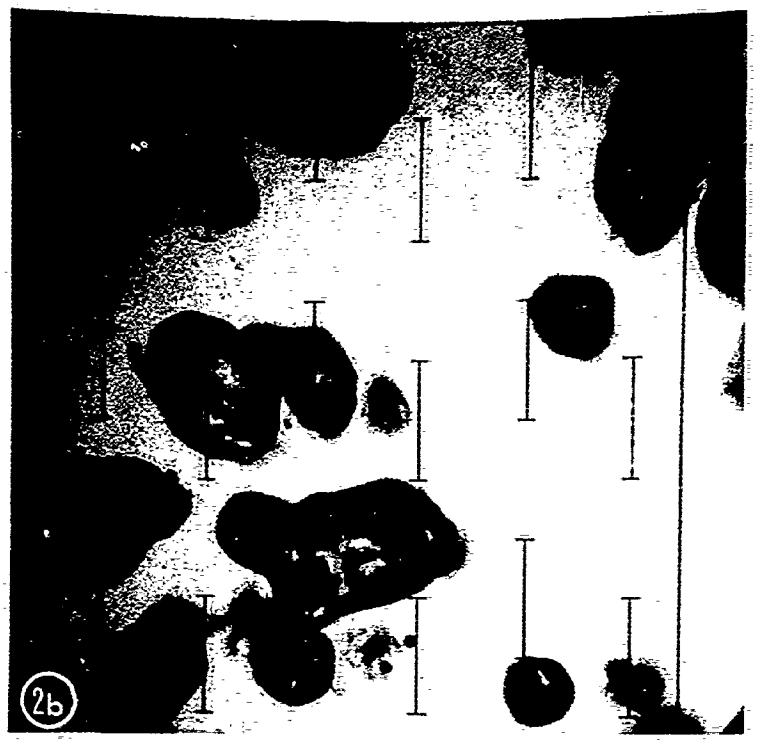
15

Figure 1: Gross photograph of a placenta in "toxemia" of pregnancy injected with a latex solution (Ward) to show the vascular tree (arteries in blue, veins in red).


1

Figure 2, a and b: a) Microscope with a reticle screen attached to the head was used for the determination of the number of syncytial sprouts.

b) A placental section with clearly illustrated villi and syncytial sprouts is projected on the screen.
Magnification = X 140


2b

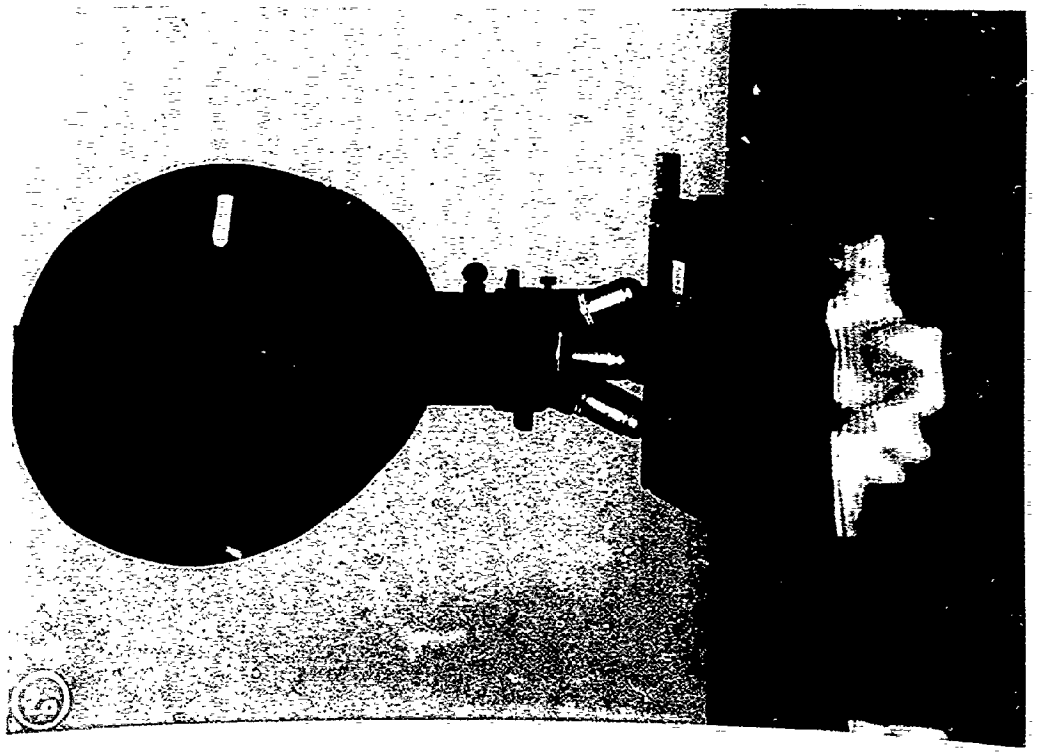
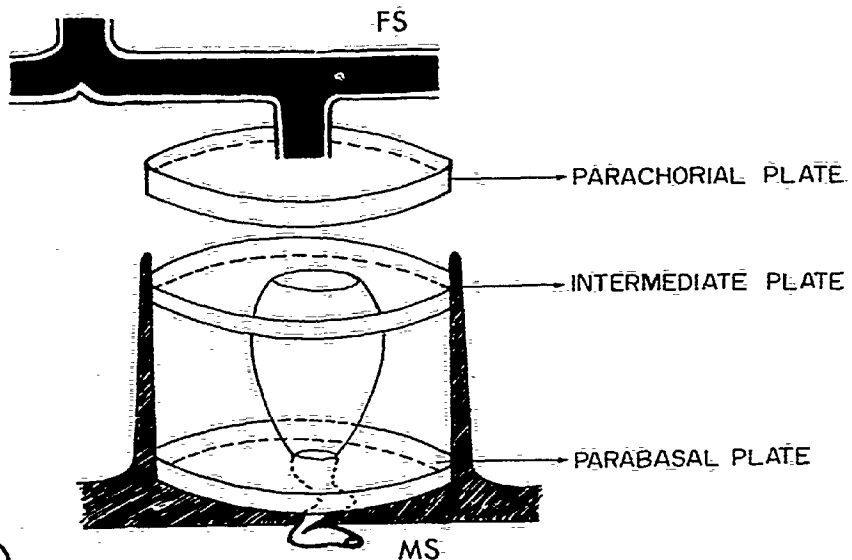
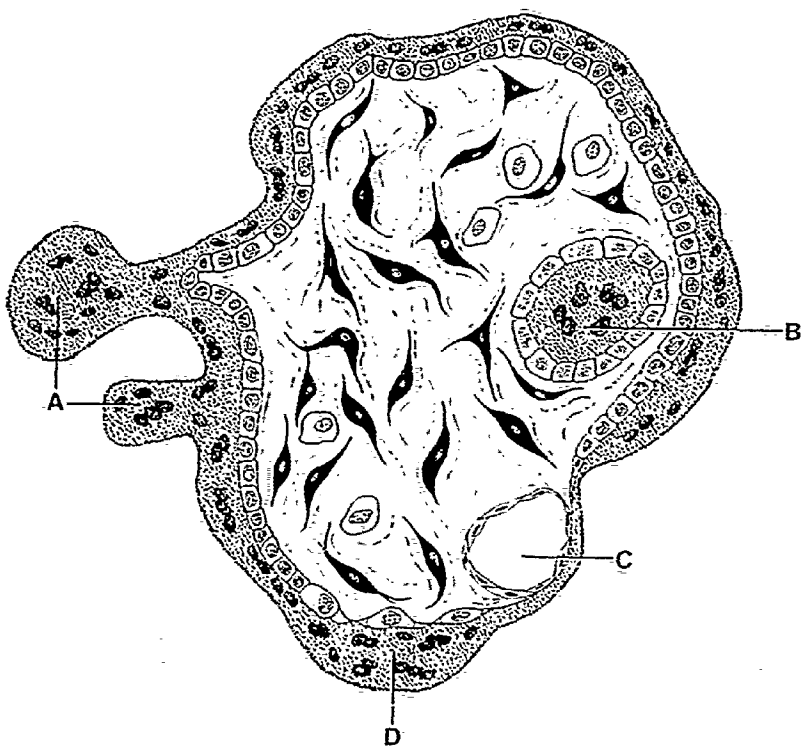


Figure 3: Schematic representation of different plates (=levels) of the fetal cotyledon (parachorial, intermediate and parabasal) that are related to the blood pO_2 gradient operating in the intervillous space (Modified from Alvarez et al., Am J Obstet Gynecol 106: 416, 1970).
FS = Fetal surface of the placenta.
MS = Maternal surface of the placenta.


FS
PARACHORIAL PLATE
INTERMEDIATE PLATE
PARABASAL PLATE
MS

3

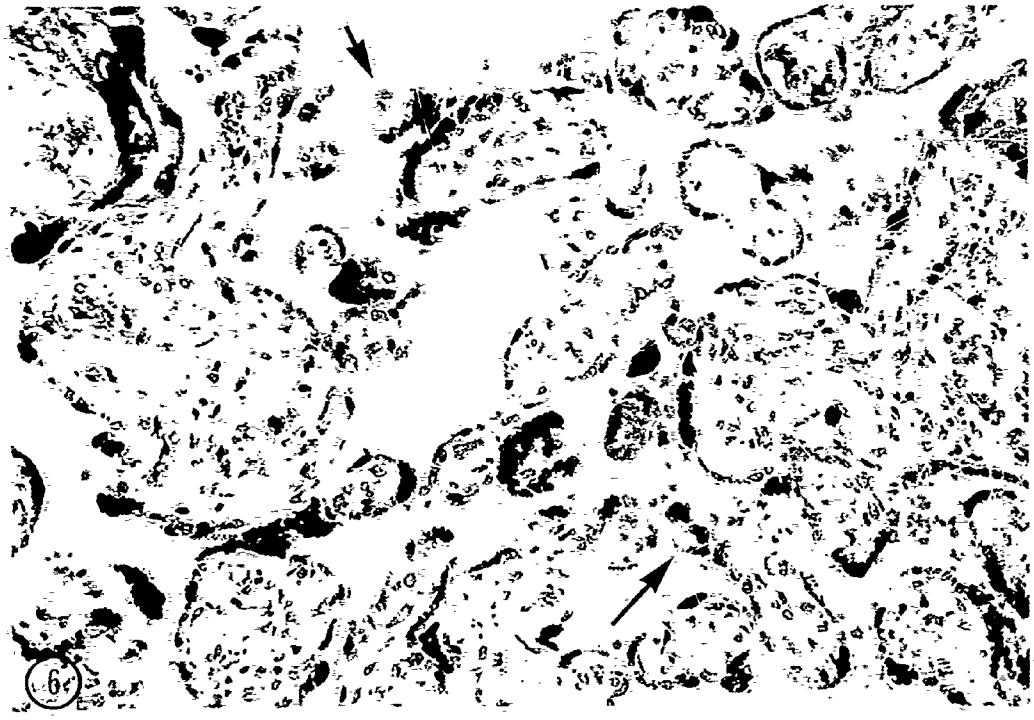
Figure 4: Schematic representation of the syncytial derivatives of a chorionic villus.
A) two different stages in the development of a syncytial sprout;
B) a stromal trophoblastic bud;
D) a syncytial knot or clump close to a capillary (C) (Modified from Boyd and Hamilton, The Human Placenta, The Macmillan Press Ltd., p. 182, 1970).



④

Figures 5 and 6: A chorionic villus of a "toxemic" placenta showing several syncytial sprouts projecting into the inter-villous space (arrows). The sprouts show numerous trophoblastic nuclei and their villus attachments are constricted into a stalk-like manner. They are predominantly attached to the lateral aspect of the villous surface.
Masson's Trichrome;
Magnification = X 160

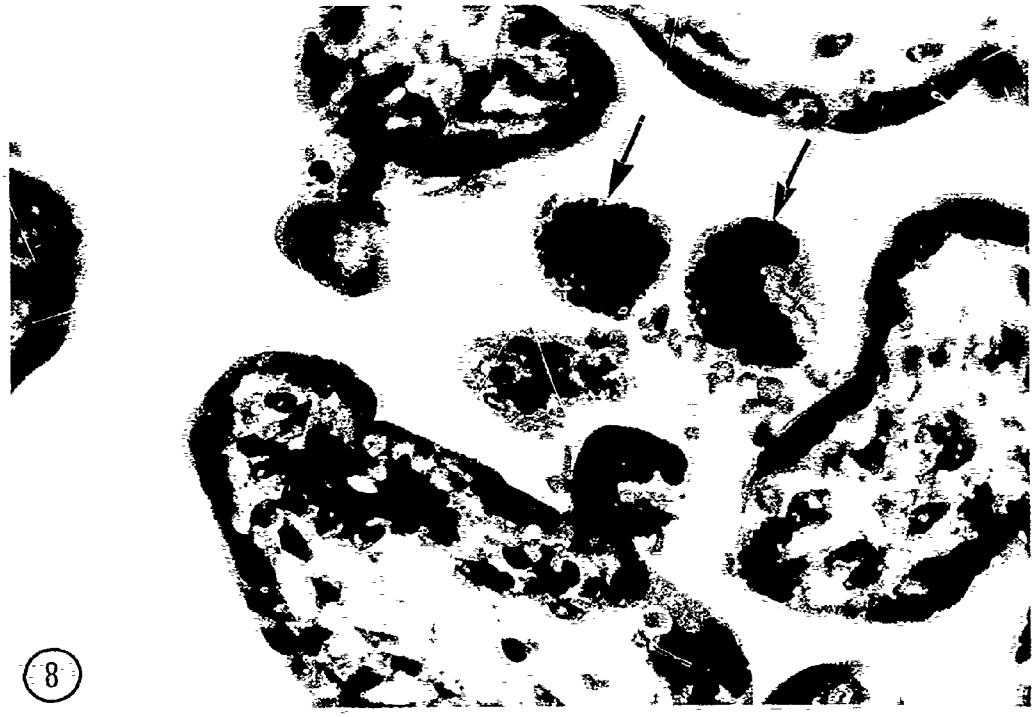
5



6

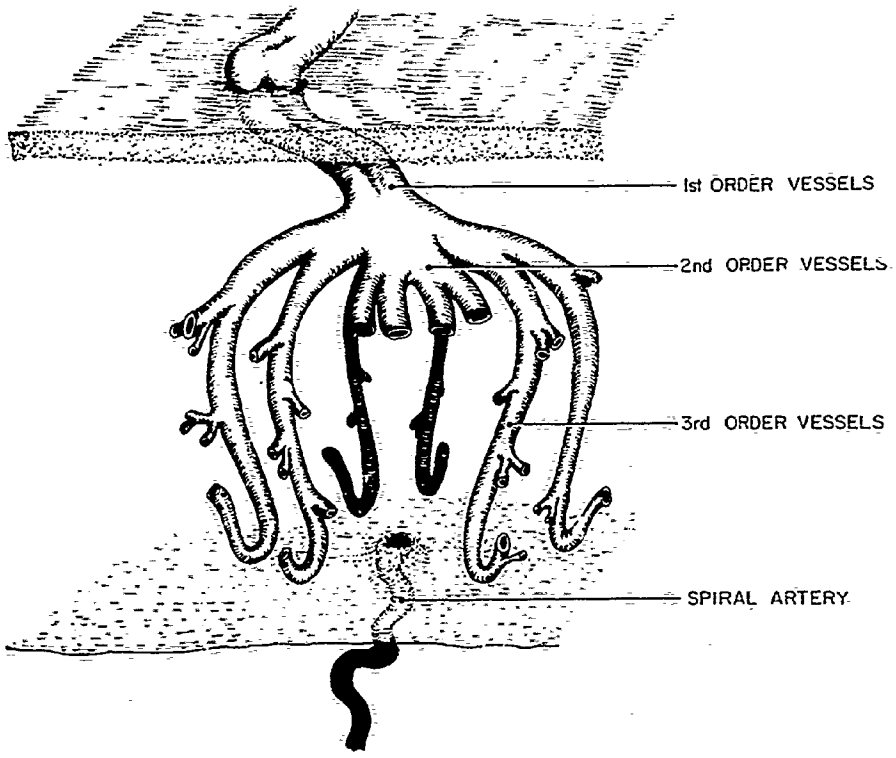
Figure 7: Photomicrograph of developing syncytial sprouts in a "toxemic" placenta. These syncytial masses are at first represented by accumulations of nuclei within a definite and circumscribed area (a). This area becomes raised, and subsequently forms a sprout which becomes elongated and is attached to the villus by a peduncle (b).
Masson's Trichrome;
Magnification = X 256

Figure 8: High magnification of a chorionic villus in a "toxemic" placenta showing a few syncytial sprouts. They are cylindrical, club-shaped with well-stained nuclei without any signs of degeneration. Cytotrophoblastic cells and mesodermal tissue cannot be distinguished in the sprouts. "Free" syncytial sprouts are seen in the intervillous space (arrows).
Masson's Trichrome;
Magnification = X 410



(8)

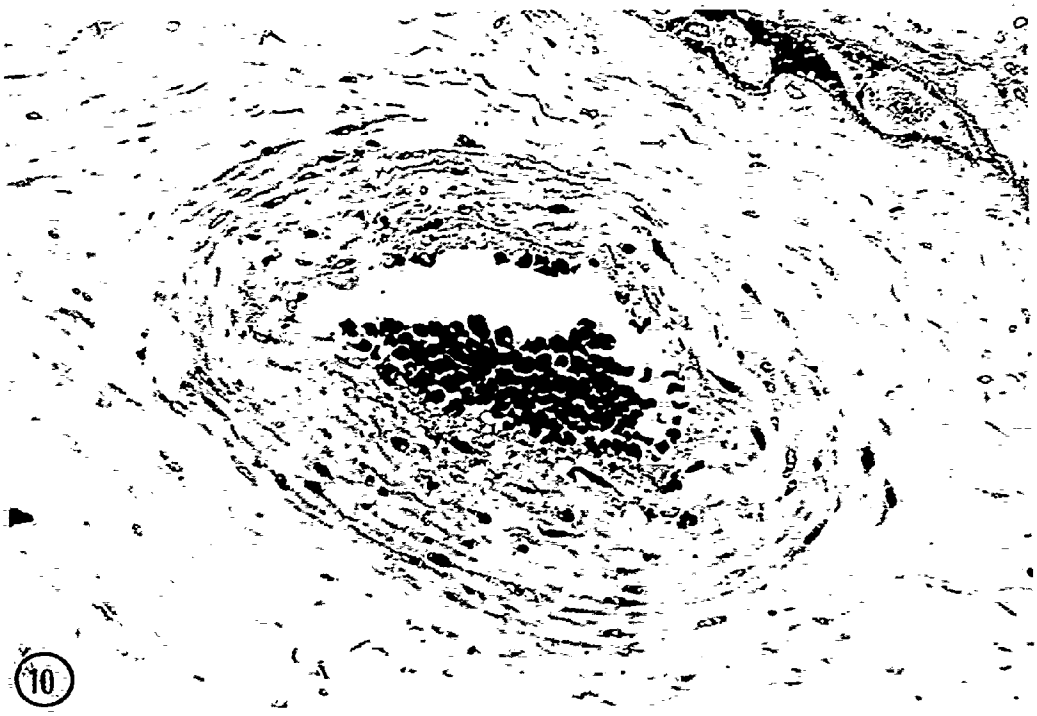
Figure 9: A schematic drawing to illustrate the distribution of the cotyledonary vessels. A vessel of first order reaches the fetal cotyledon; as the 1st order vessel enters the cotyledon it divides into a number of secondary vessels (2nd order) and these divide once again into the 3rd order vessels.


1st ORDER VESSELS
2nd ORDER VESSELS
3rd ORDER VESSELS
SPIRAL ARTERY

(9)

Figure 10: Normal fetal stem artery. General view of a transverse section showing the arrangement of the layers of the arterial wall. The adventitia proper is missing. Between the medial layer of the artery and the surrounding connective tissue there is a sharply defined border. The lumen is widely open and partially filled with red blood cells.
Toluidine Blue; Magnification = X 256

Figure 11: Normal fetal stem artery. Higher magnification of a portion of the artery wall to illustrate an intact and simple endothelium (e), a relatively thick media (m) consisting of smooth muscle cells intermingled with collagenous fibrils. The distribution of the muscle cells is largely circular in the outer part of the media and longitudinal or spiral towards the endothelium.
Masson's Trichrome; Magnification = X 410



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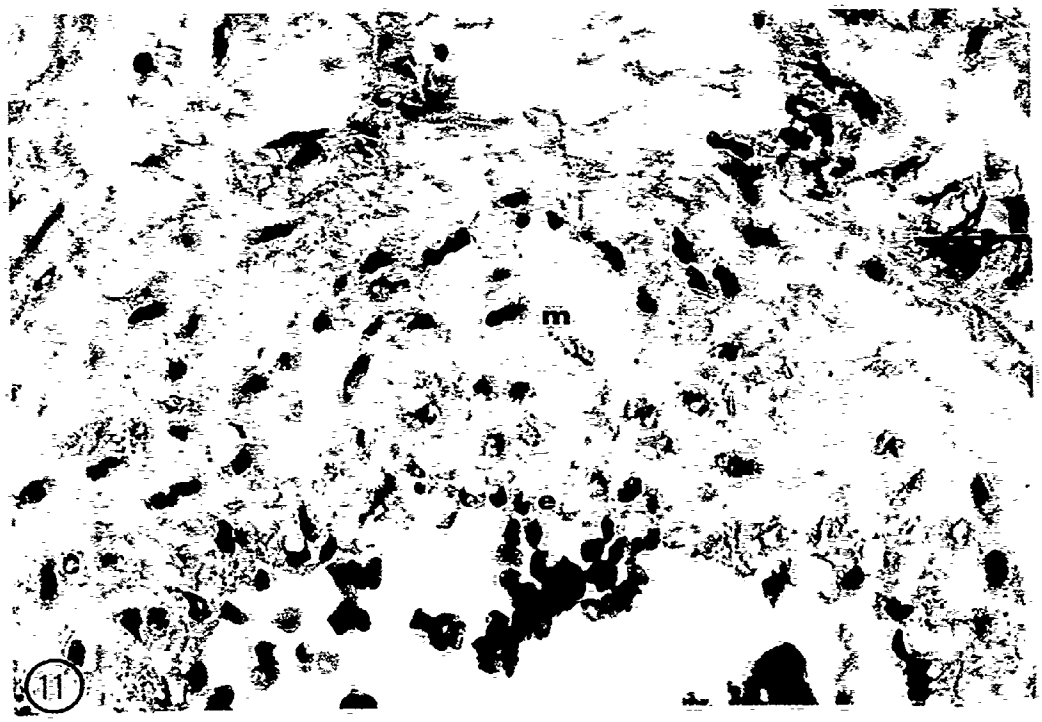
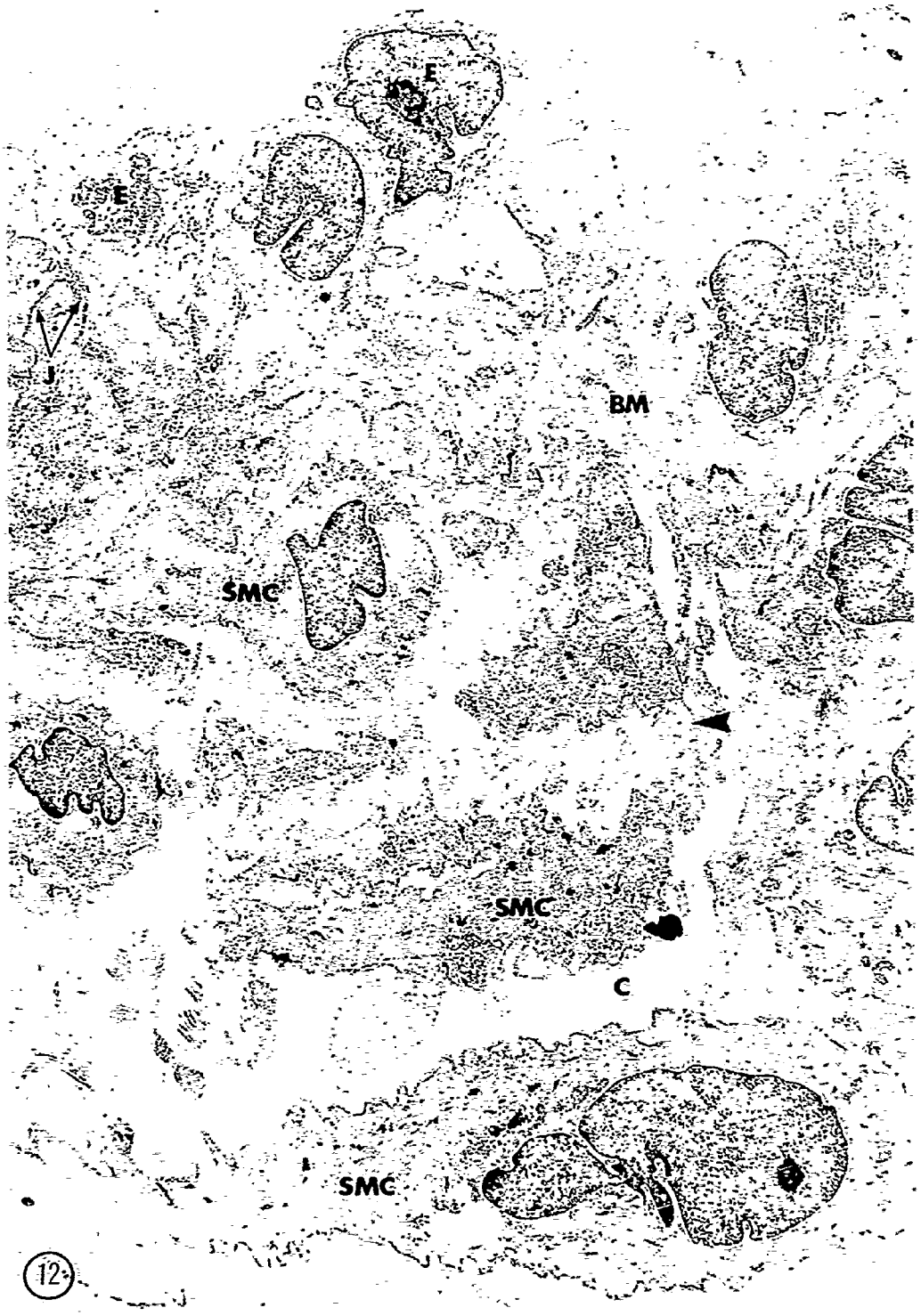


11

Figure 12: Detail of the intima and inner media of a normal fetal stem artery of 3rd order. The endothelial cells (E) project into the lumen and at their basal portion form intercellular junctions (J) with each other. The smooth muscle cells (SMC) have a circular, spiral and longitudinal arrangement depending on the layer of the media. Collagen bundles (C) and basement membrane (BM) are present in the interstitium as are numerous small cell processes. Similar cell processes are occasionally seen attached to the smooth muscle cells (arrow). Magnification = X 7,250.



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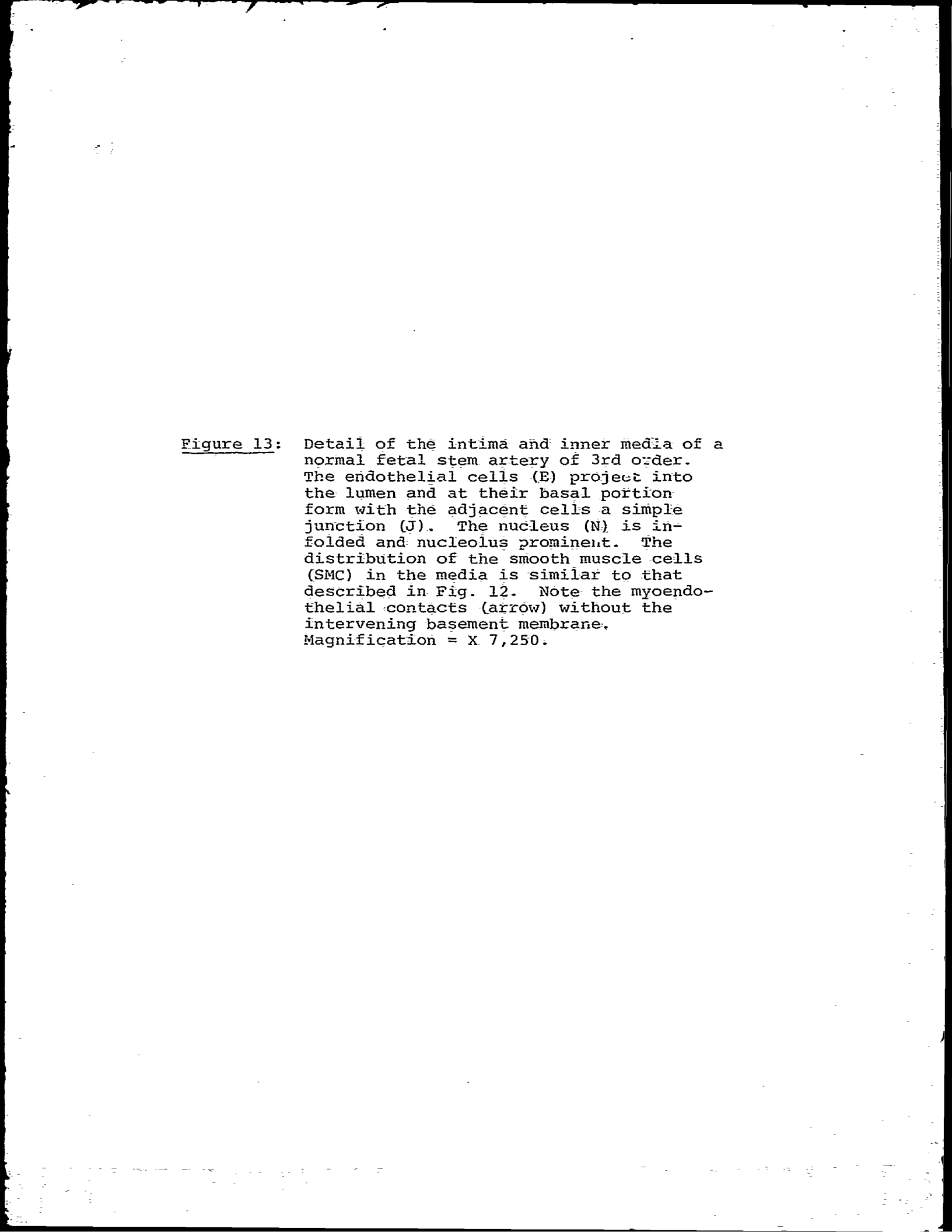
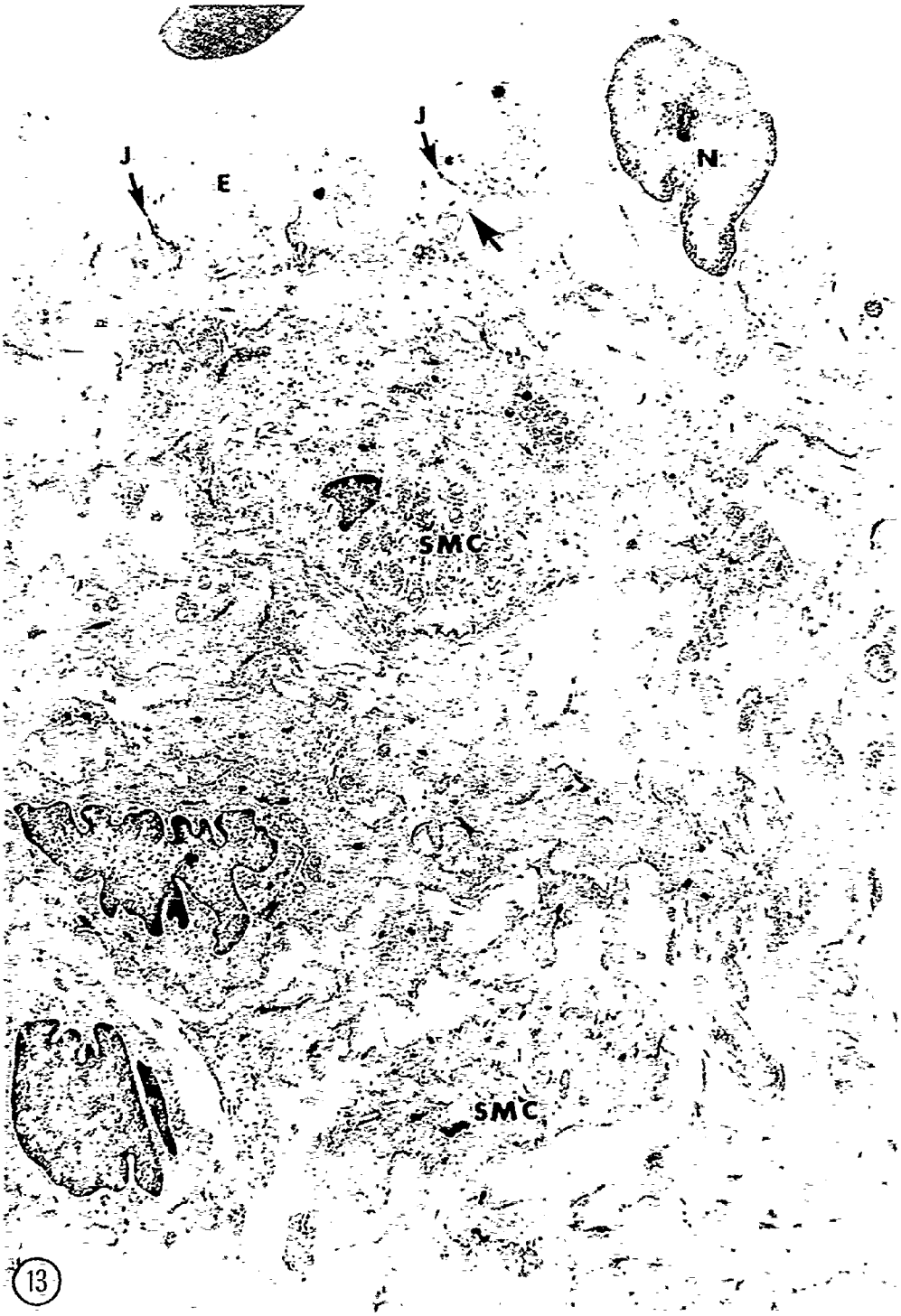


Figure 13: Detail of the intima and inner media of a normal fetal stem artery of 3rd order. The endothelial cells (E) project into the lumen and at their basal portion form with the adjacent cells a simple junction (J). The nucleus (N) is infolded and nucleolus prominent. The distribution of the smooth muscle cells (SMC) in the media is similar to that described in Fig. 12. Note the myoendothelial contacts (arrow) without the intervening basement membrane. Magnification = X 7,250.


J
E
J
N
SMC
SMC
13

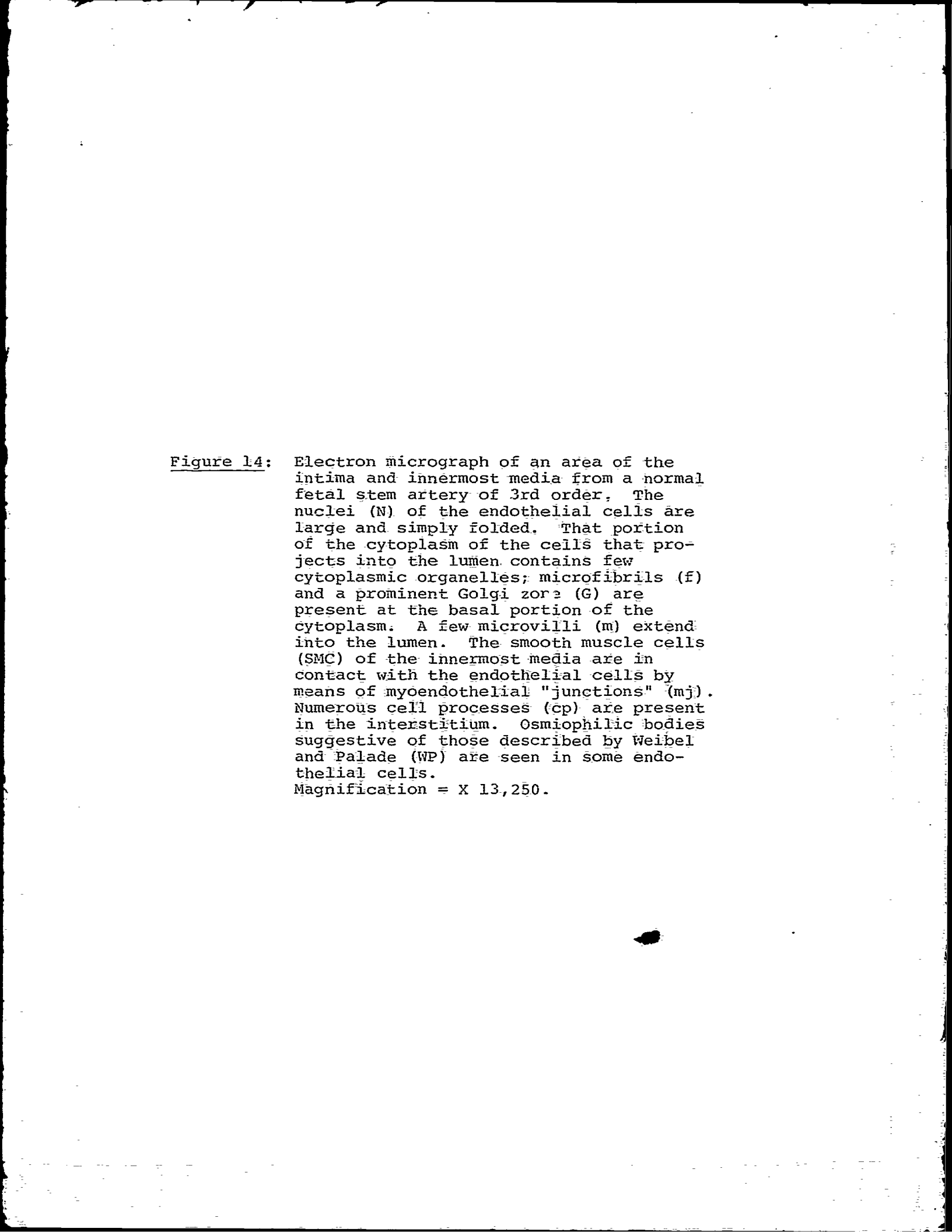


Figure 14: Electron micrograph of an area of the intima and innermost media from a normal fetal stem artery of 3rd order. The nuclei (N) of the endothelial cells are large and simply folded. That portion of the cytoplasm of the cells that projects into the lumen contains few cytoplasmic organelles; microfibrils (f) and a prominent Golgi zone (G) are present at the basal portion of the cytoplasm. A few microvilli (m) extend into the lumen. The smooth muscle cells (SMC) of the innermost media are in contact with the endothelial cells by means of myoendothelial "junctions" (mj). Numerous cell processes (cp) are present in the interstitium. Osmiophilic bodies suggestive of those described by Weibel and Palade (WP) are seen in some endothelial cells.
Magnification = X 13,250.

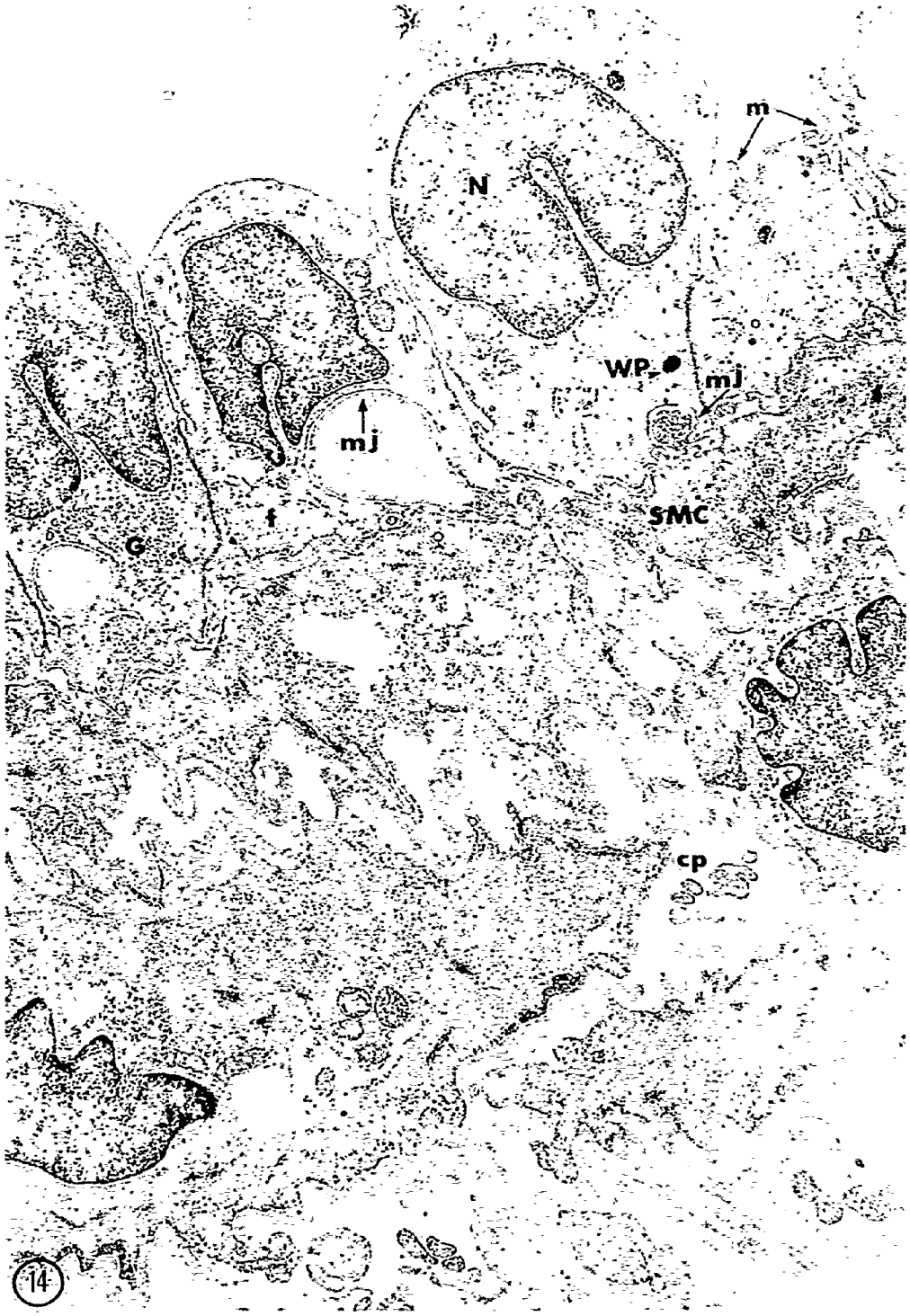

m
N
WP
mj
mj
SMC
f
G
cp
14

Figure 15: Normal fetal stem artery of 3rd order. The intima shows prominent endothelial cells (E) with large nuclei (N) and prominent nucleoli (n). The chromatin has a distribution characteristic for the endothelial cells. In the media, the smooth muscle cells (SMC) are of the complex branching variety and arranged in circumferential and longitudinal fashion.
Magnification = X 7,250.

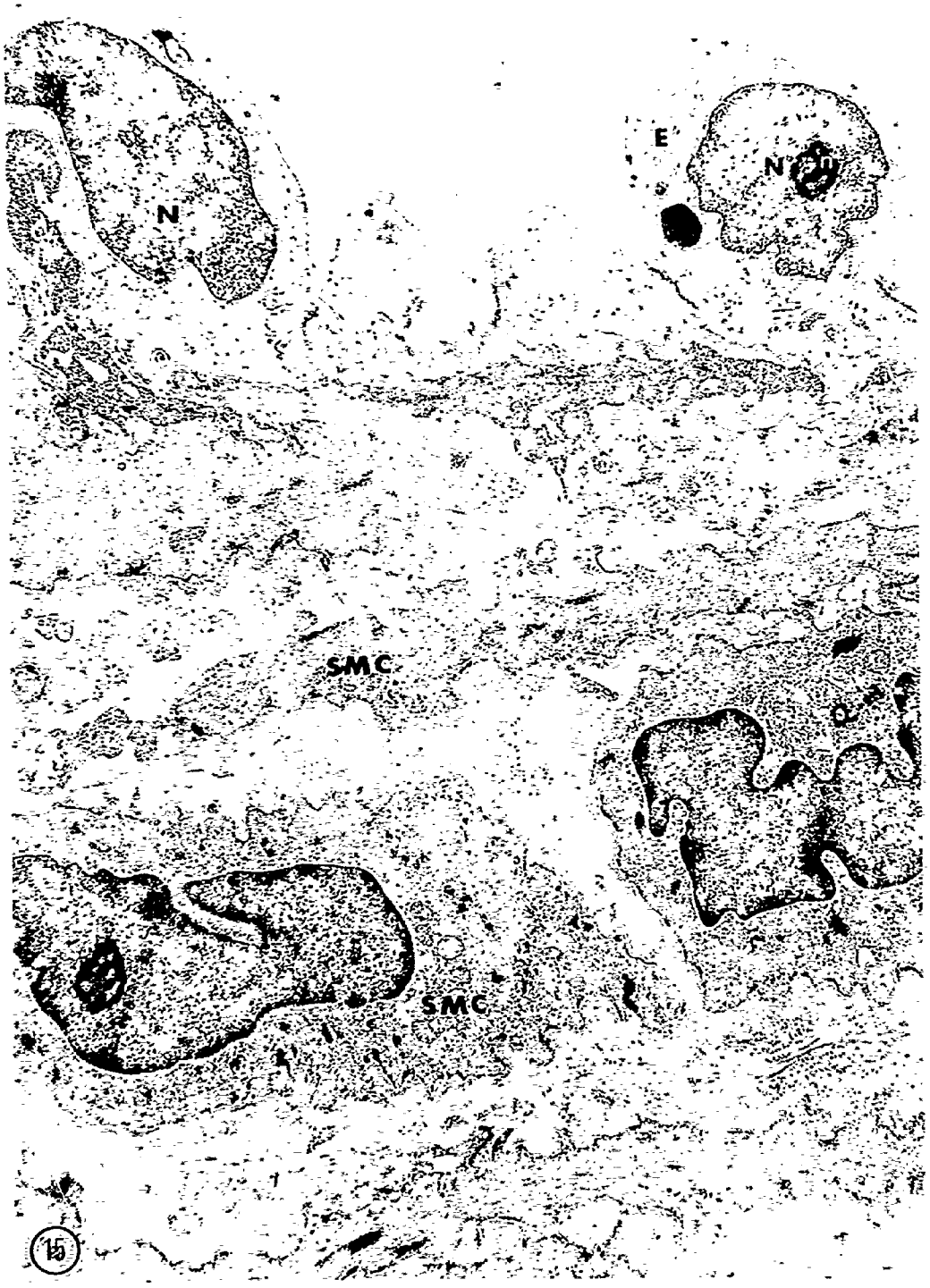

N
E
N
SMC
SMC
15

Figure 16: Detail of the intima and inner media of a normal fetal stem artery of 3rd order. One of the endothelial cells (E) is seen in mitosis (M) and the junctions (J) of this cell with the adjacent ones are of considerable length. The basement membrane (BM) of the endothelial cells shows different thickness and, sometimes, areas of reduplication or aggregation. Myo-endothelial junctions (mj) are formed between the endothelial and smooth muscle cells. Small cell processes extend occasionally from the smooth muscle cells into the interstitium (arrow). Magnification = X 16,250.

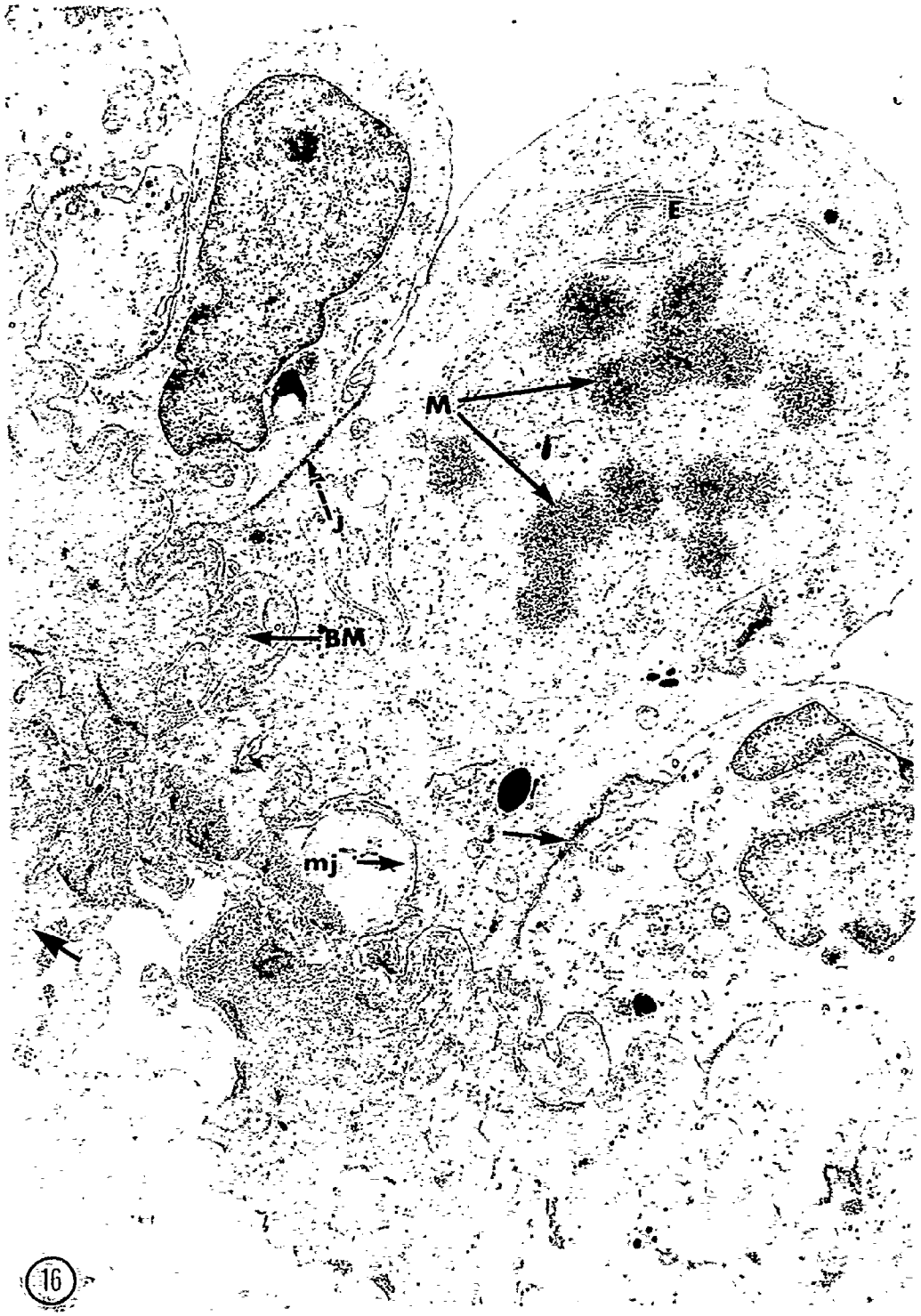

E
M
BM
mj
16

Figure 17: Electron micrograph of an area of the intima and inner media from a normal fetal stem artery of 3rd order. The intima shows endothelial cells (E) whose main bodies project into the lumen. The cellular processes of the smooth muscle cells (SMC) in contact with the endothelial cells appeared swollen (arrows). Several endothelial cells contain small osmiophilic bodies reminiscent of Weibel-Palade bodies.
Magnification = X 7,250.

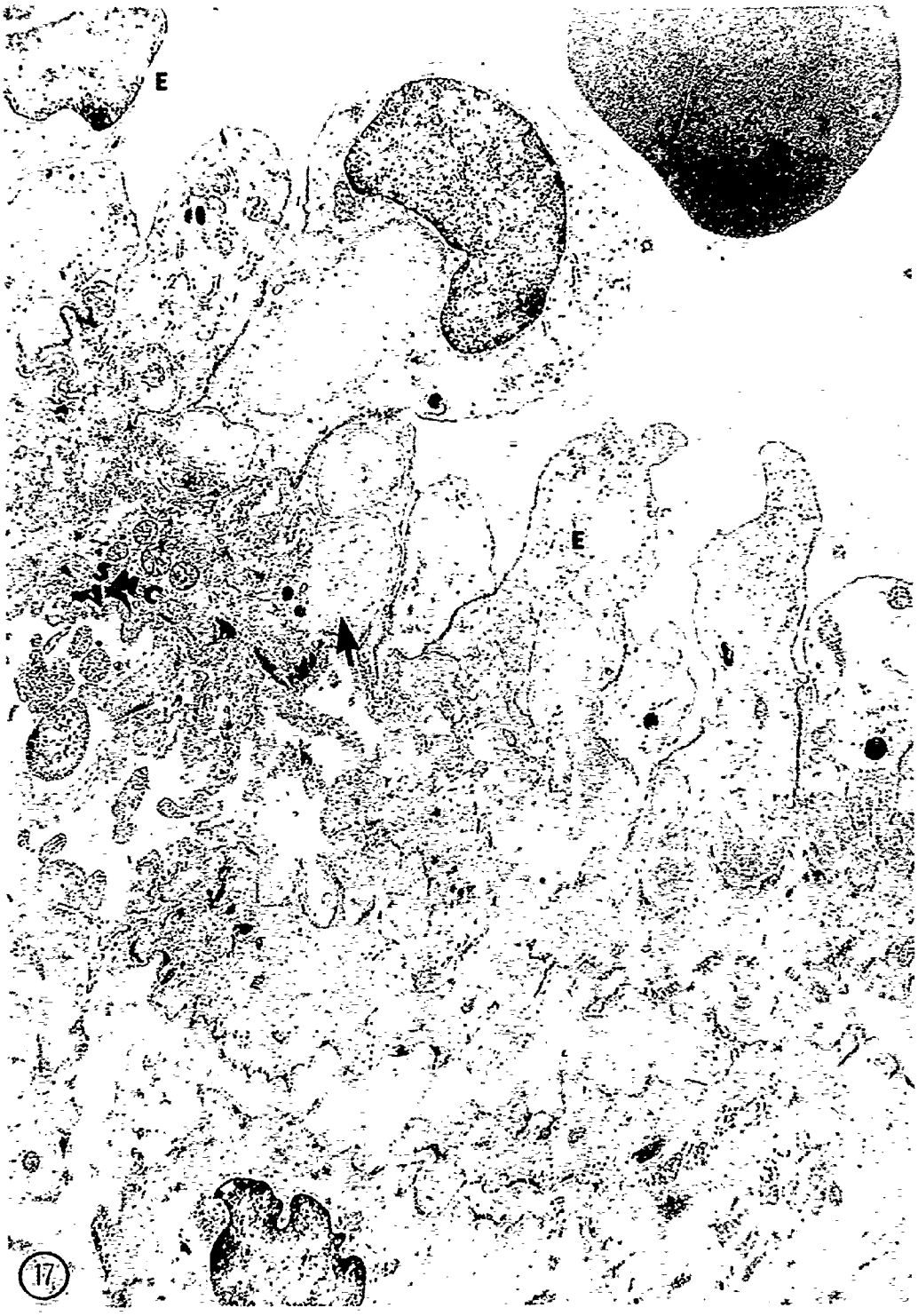

E
E
17

Figure 18: Detail of the intima and inner media of a normal fetal stem artery of 3rd order. One of the endothelial cells (E) shows a large, folded nucleus (N). The process of a smooth muscle cell (SMC) at one of the myoendothelial junctions is slightly swollen (arrow). The interstitial spaces between the smooth muscle cells are occupied by collagen fibrils (C), basement membrane substance (BM) and numerous small cell processes (cp). A similar process is attached to a smooth muscle cell (double arrow).
Magnification = X 10,600.

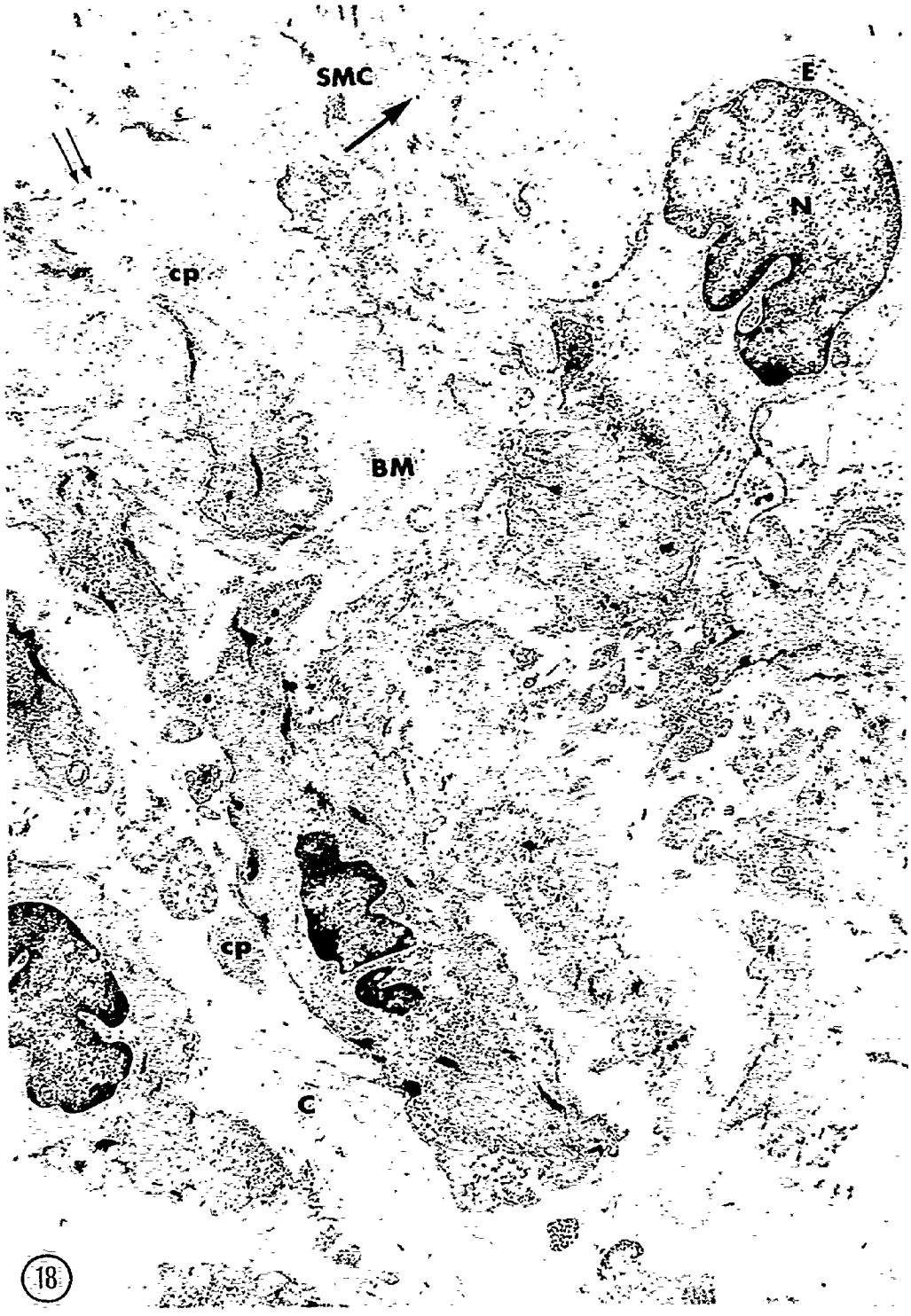

SMC
E
N
cp
BM
cp
C
18

Figure 19: Intima and media of normal fetal stem artery of 3rd order. The interstitial spaces are occupied by bundles of collagen fibrils (C), basement membrane (BM) and cellular processes (arrow). These processes do not contain recognizable cytoplasmic organelles and are not surrounded by a basement membrane. Magnification = X 7,250.

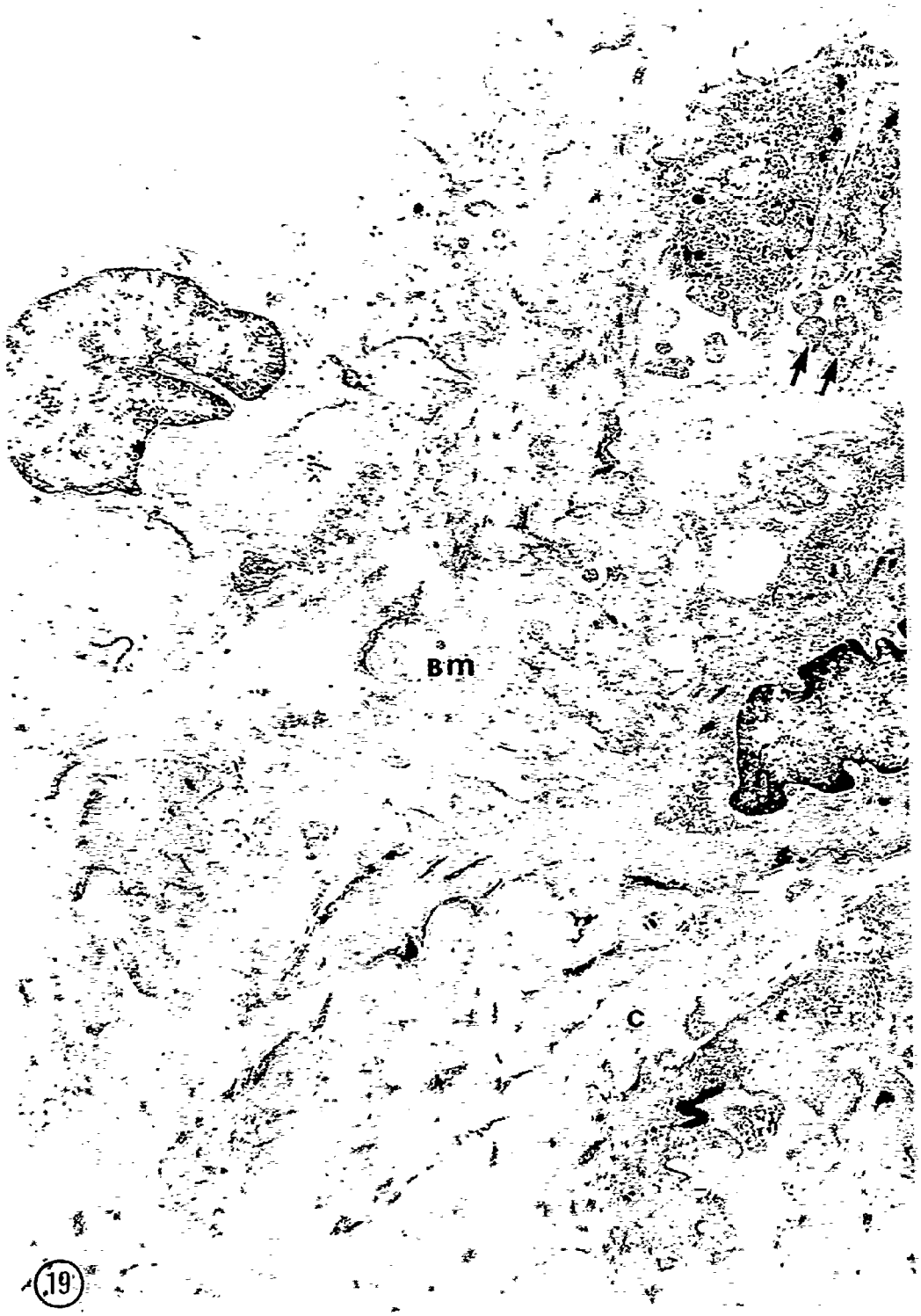
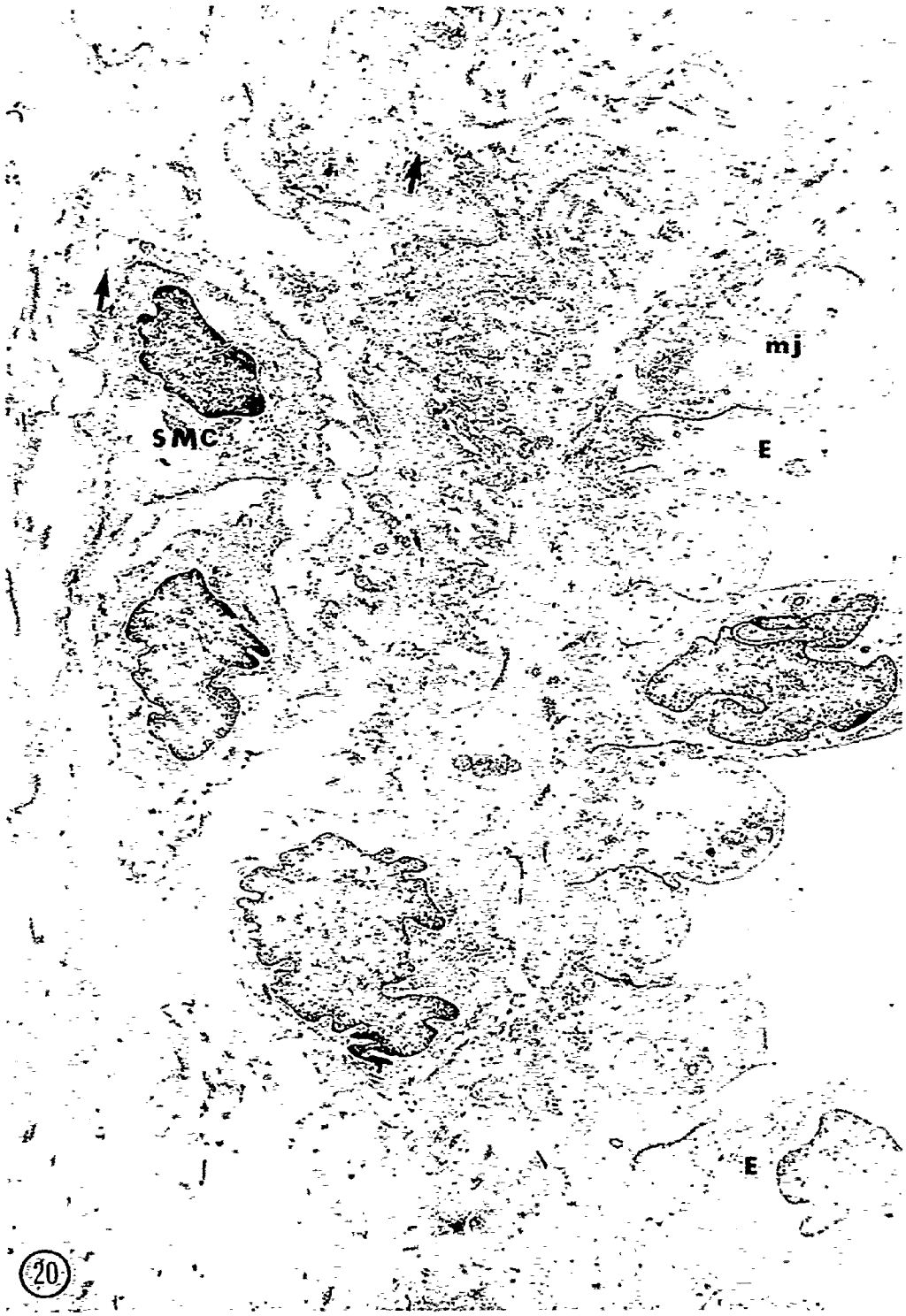
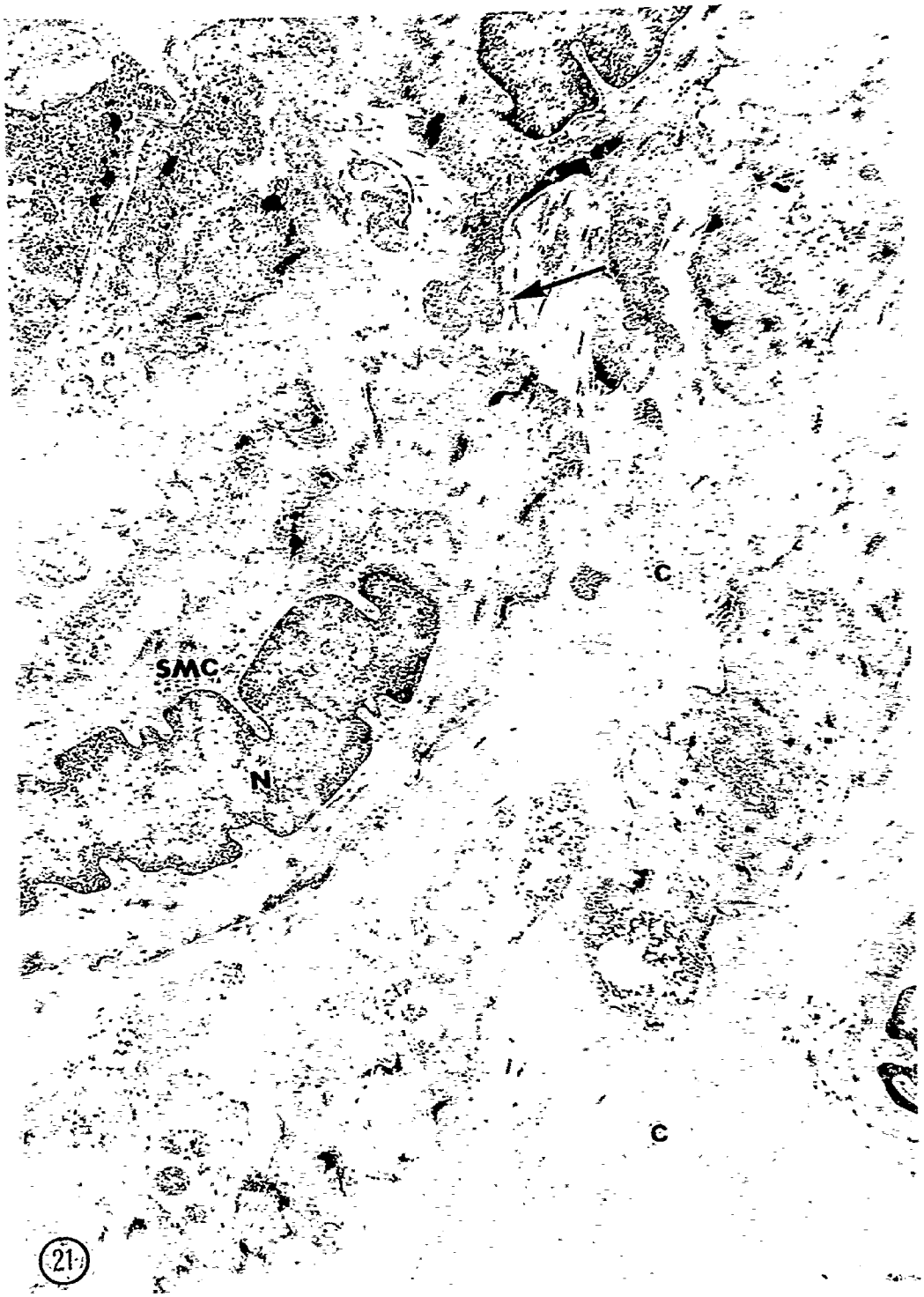

Bm
C
19

Figure 20: Electron micrograph of the intima and the innermost media from a normal fetal stem artery of 3rd order. The nuclei of the medial smooth muscle cells (SMC) have a typical fence-like contour and a characteristic chromatin distribution. Some of the cellular processes extending from the main body of the smooth muscle cells (arrows), and processes contacting the endothelial cells (E) at the myoendothelial "junctions" (mj) appear slightly swollen.
Magnification = X 7,250.



20

Figure 21: Detail of the inner media of a normal fetal stem artery of 3rd order. One of the medial smooth muscle cells (SMC) has a centrally placed nucleus (N) with a fence-like contour. The cytoplasm contains a few organelles and is largely occupied by myofilaments; it shows triangular and oblong densities. There is widening of the intercellular spaces towards the adventitia. Some small cellular processes extend from the smooth muscle cells (arrow).
Magnification = X 7,250.



21

Figure 22: Fetal stem artery from a placenta in "toxemia" of pregnancy. A transverse section to illustrate the swelling and proliferation of the endothelial cells which produce luminal narrowing. Sub-endothelial cellular proliferation is also present (arrow).
Masson's Trichrome; Magnification = X 252

Figure 23: Fetal stem artery from a placenta in "toxemia" of pregnancy. Higher magnification showing endothelial proliferation simulating intra-arterial septa. The lumen is partially occluded by the endothelial "bridges".
Masson's Trichrome; Magnification = X 410

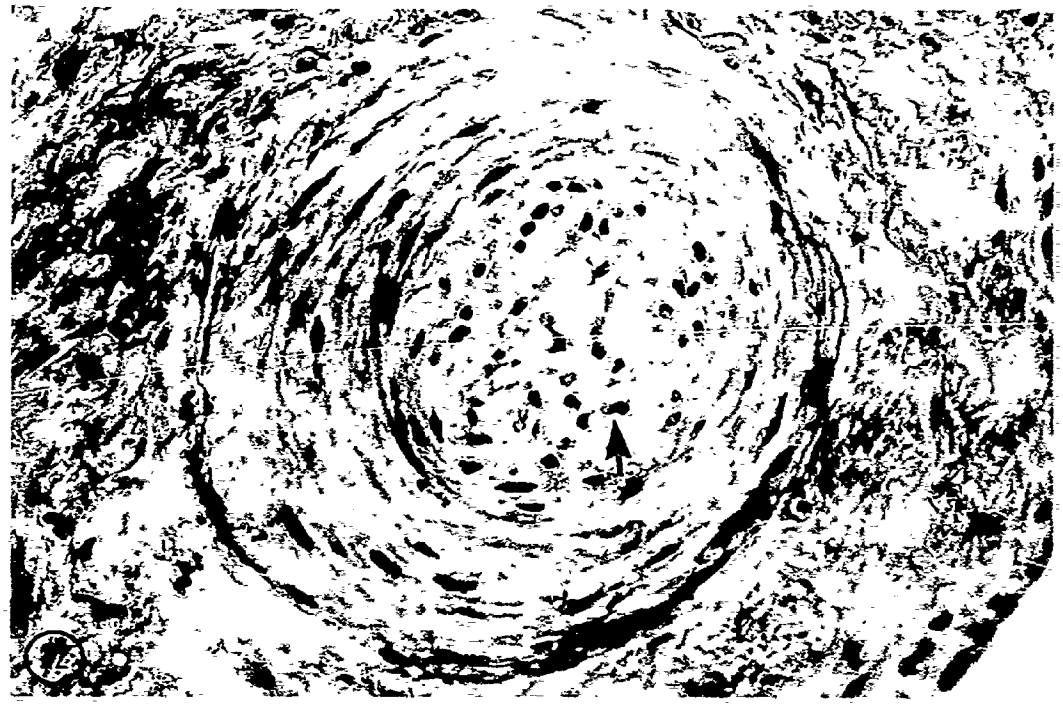
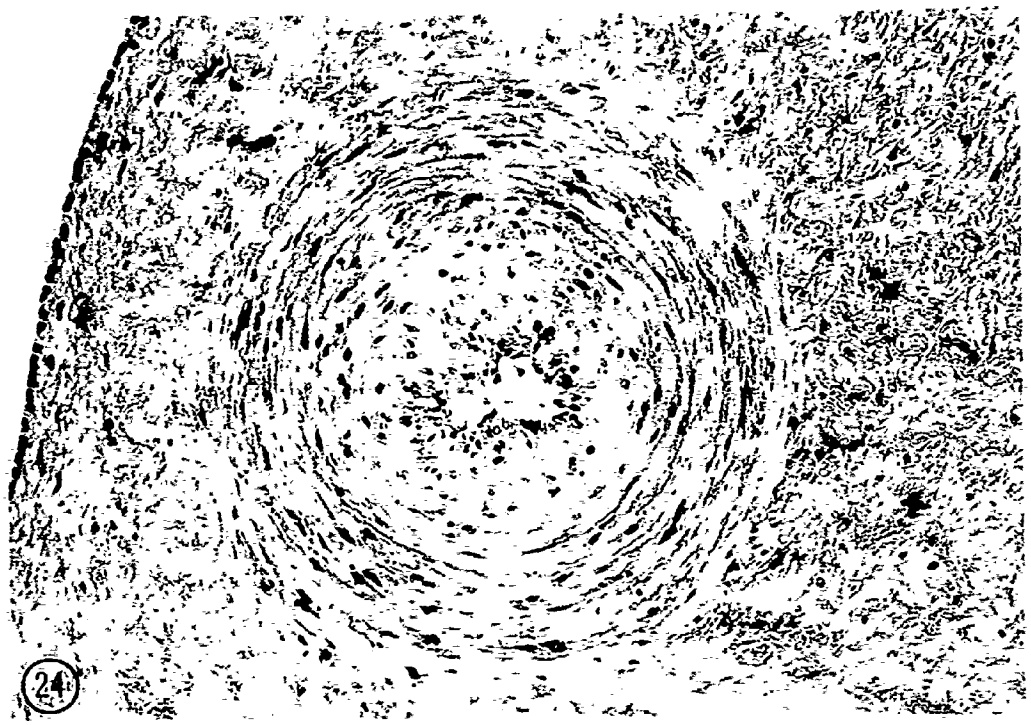


Figure 24: Transverse section of a fetal stem artery from a placenta in a "toxemic" patient. There is a medial thickening owing to the proliferation of the smooth muscle cells and fibrous tissue. The outermost layer of the arterial wall consists almost entirely of thick loose circular musculature. In the intermediate layer between the endothelial cells and the muscular layer there are proliferating subendothelial cells. The lumen is almost occluded by endothelial proliferation. The border between the arterial wall and the surrounding connective tissue is well defined.
Masson's Trichrome; Magnification = X 256

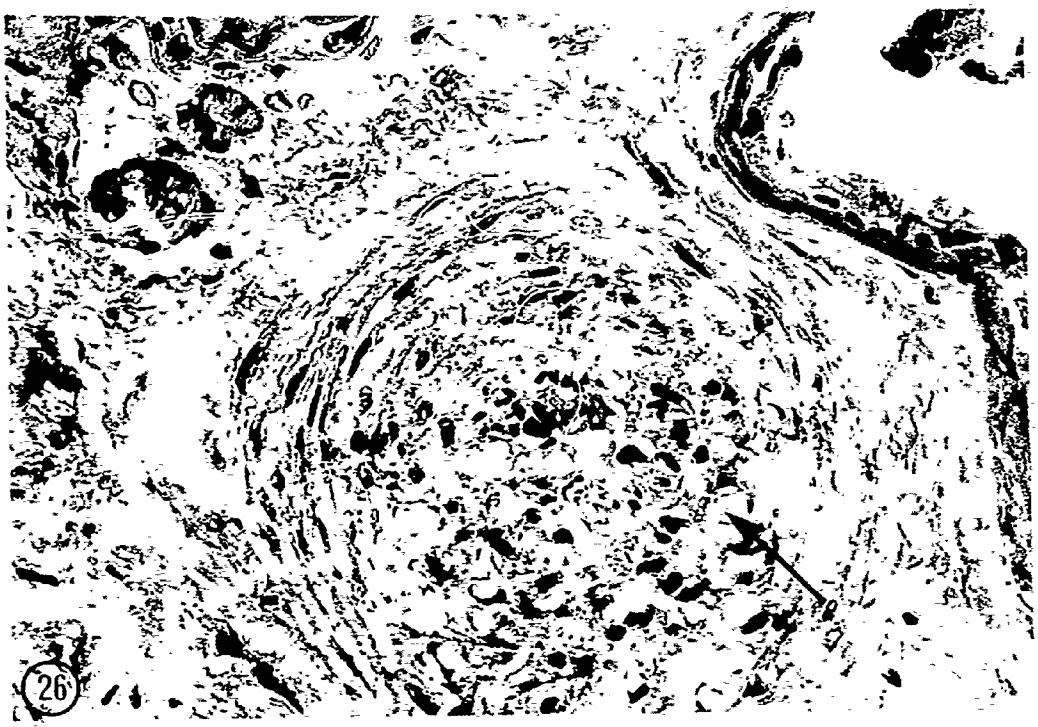
Figure 25: Transverse section of a fetal stem artery from a placenta in a "toxemic" patient showing the proliferation of smooth muscle cells. Their arrangement appears to be random. There is marked vacuolation in the spaces between the smooth muscle cells. Intimal proliferation with lumen occlusion is also present.
Toluidine Blue; Magnification = X 320


24

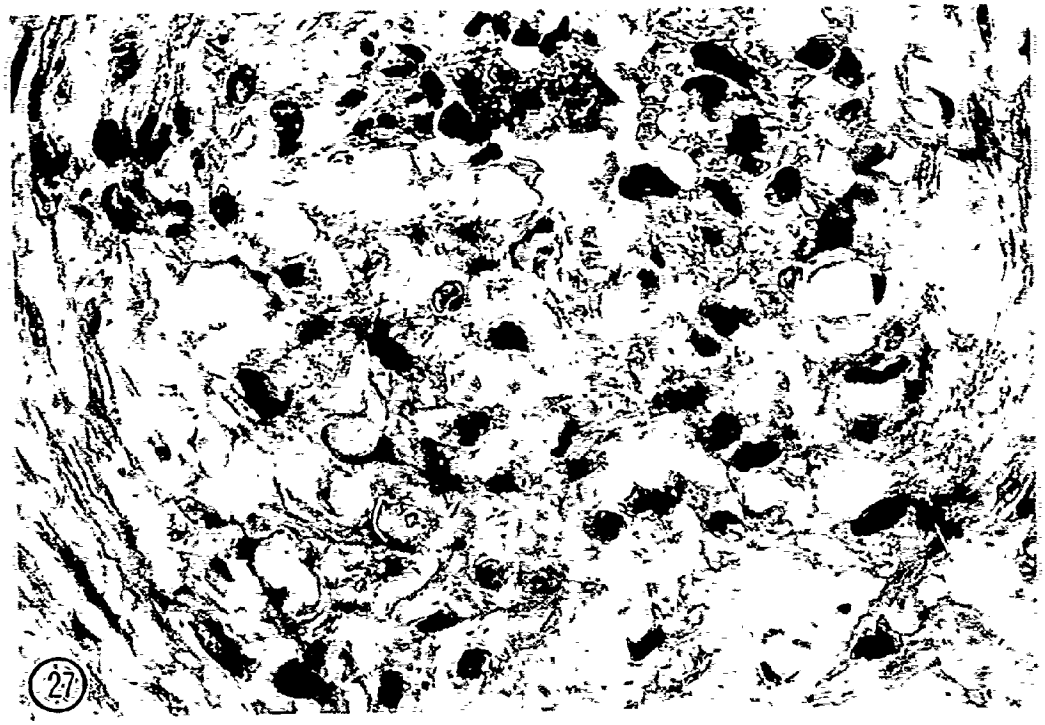

25

Figure 26: Transverse section of a fetal stem artery from a placenta in a "toxemic" patient. The smooth muscle cells are oriented circumferentially in approximated half of the artery, whereas the other half shows the muscle cells distributed haphazardly (arrow).
Masson's Trichrome; Magnification = X 256

Figure 27: High magnification of a portion of the arterial wall illustrated in Figure 26. There is loss of the circumferential orientation of the smooth muscle cells. The connective tissue intermingled with the muscle cells forms a loose network.
Masson's Trichrome; Magnification = X 410



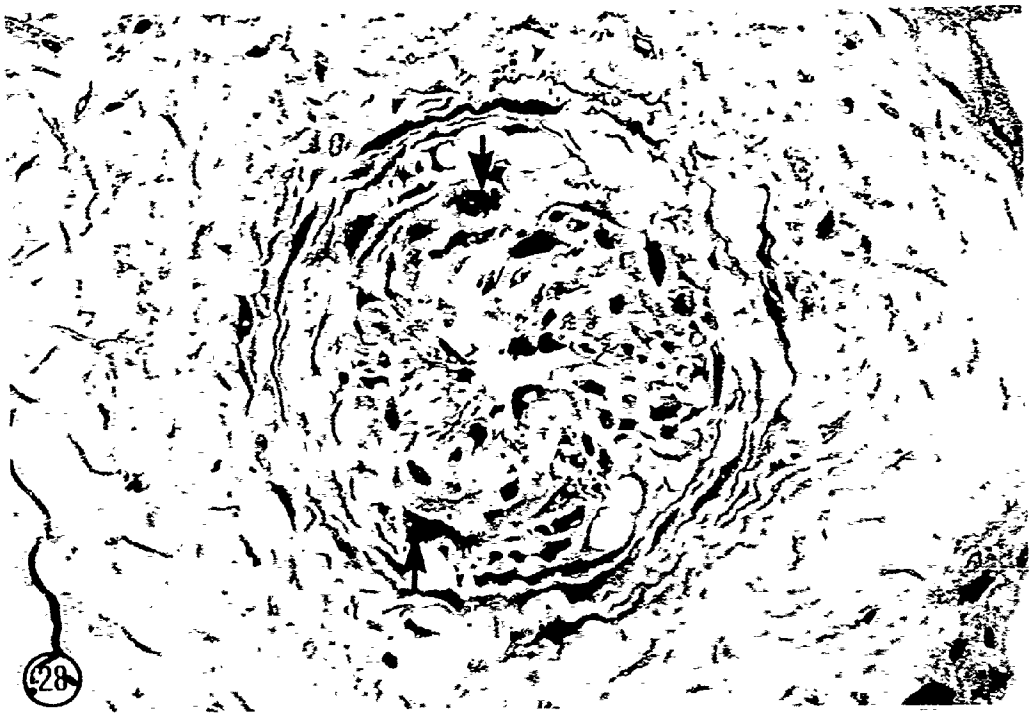
(26)



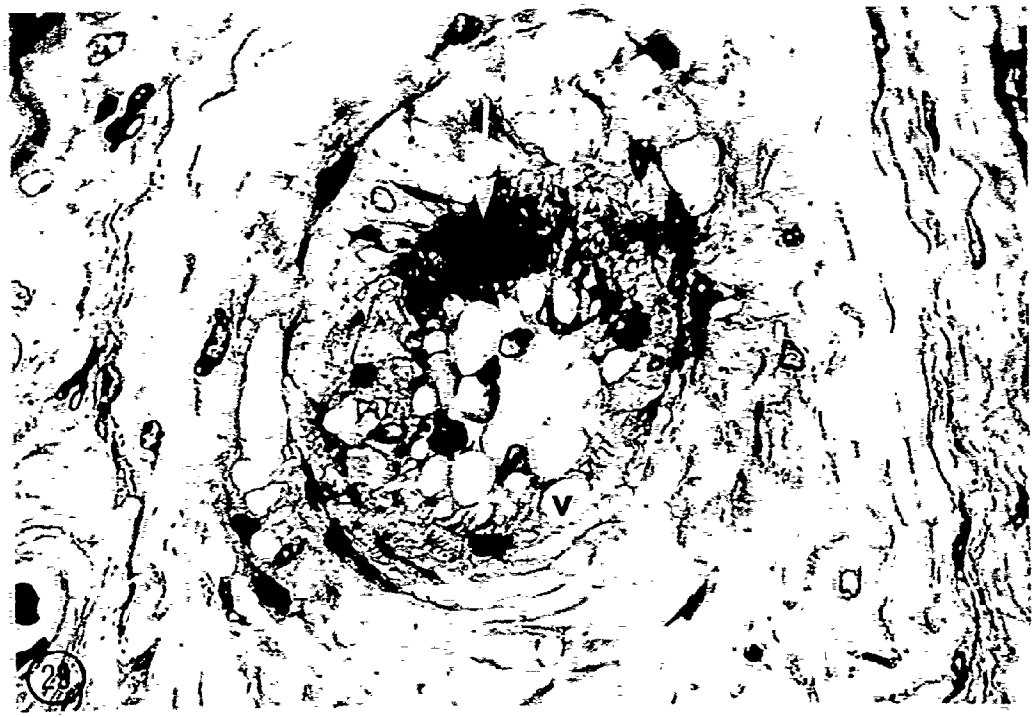
(27)

Figure 28: Photomicrograph of a transverse section of a fetal stem artery from a placenta in "toxemic" patient to show an artery with a fully developed lesion. The morphological characteristics of some smooth muscle cells in this artery are quite different from the classical spindle form; here the cells are "broad" and somewhat "bizarre" in shape (arrows).
Toluidine Blue; Magnification = X 256

Figure 29: Transverse section of a fetal stem artery from a placenta in "toxemia" of pregnancy. The well developed medial lesion with severe distortion of the smooth muscle cells. Some of these cells are filled and distended with vacuoles (v) and some appear to be in process of necrosis (arrow) with an increased basophilia of nuclei. There are also vacuoles in the spaces between the smooth muscle cells, and the endothelial cells are prominent.
Toluidine Blue; Magnification = X 252



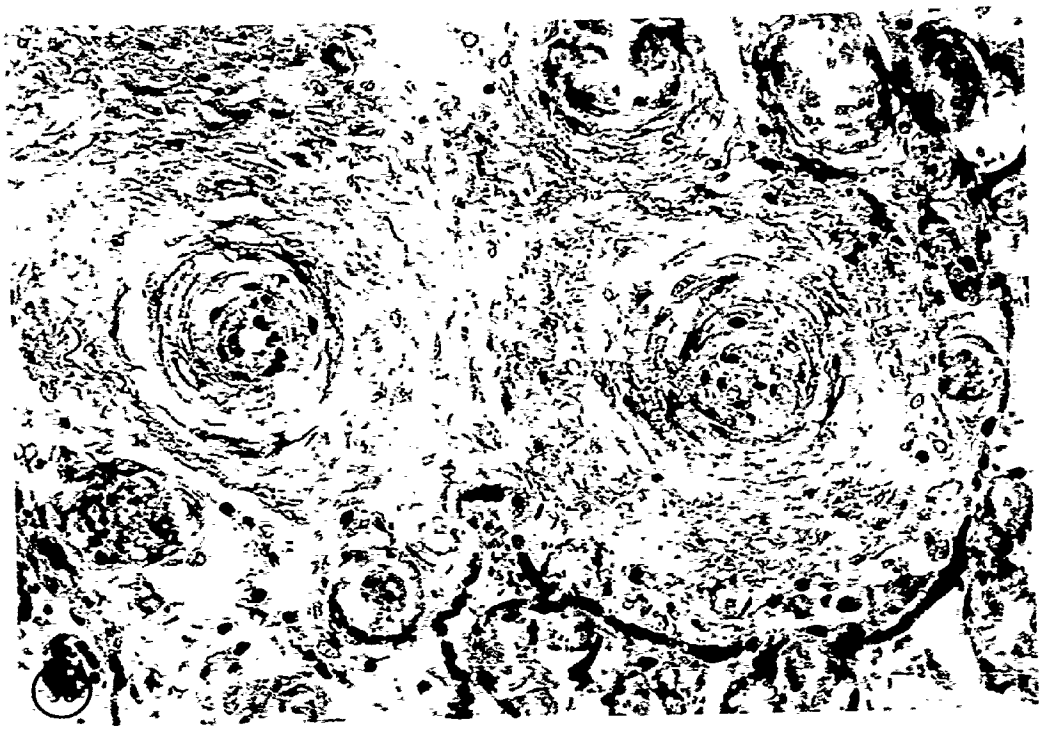
28

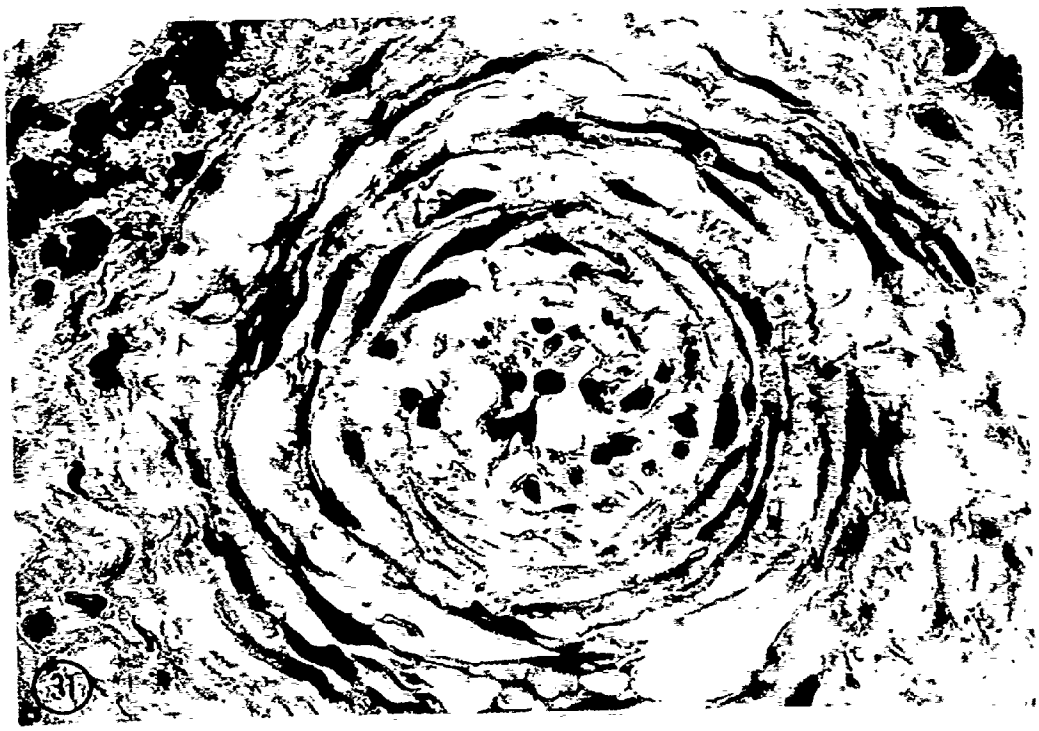


29

Figure 30: Photomicrograph of a transverse section of a ramus chorii in "toxemia" of pregnancy showing the smaller-size arteries. In these arteries the smooth muscular cells are arranged in a concentric laminar fashion, occluding almost completely the lumen. Observe that the endothelial cells do not proliferate in this type of artery.
Masson's Trichrome; Magnification = X 200

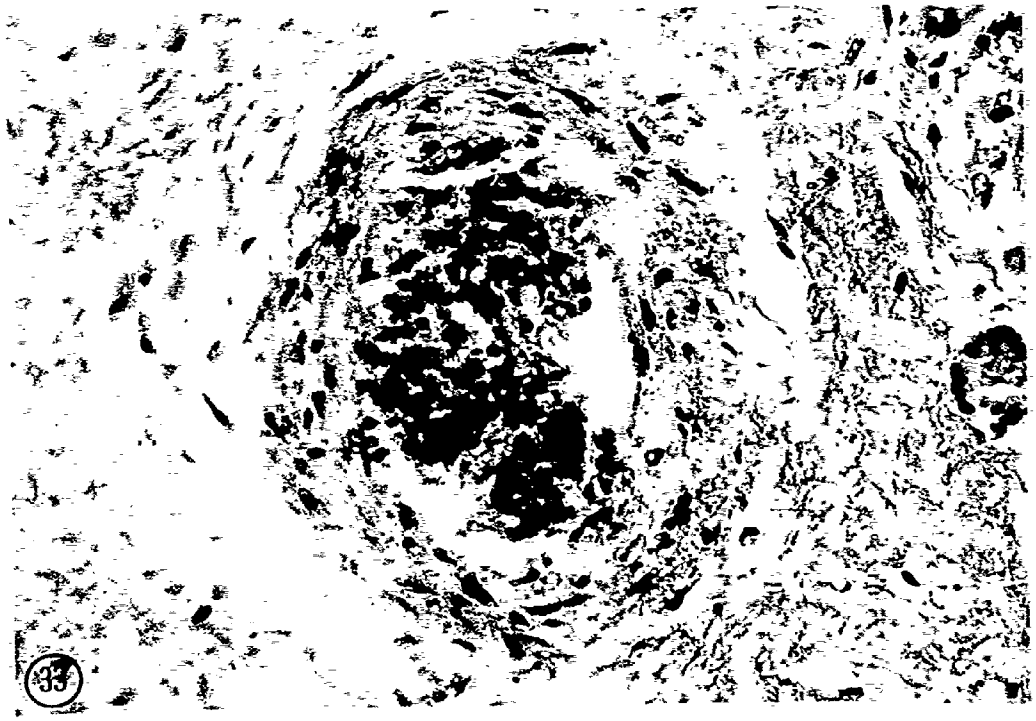
Figure 31: High magnification of a transverse section of a ramus chorii in "toxemia" of pregnancy showing the smaller-size arteries. Observe the proliferation of smooth-muscle cells, with an onion-skin type of configuration and almost obliterating the lumen. In the interspaces of the circular musculature there are individual collagen fibrils. No proliferation of the endothelial cells is seen, but cells growing from the sub-endothelial layer are present.
Masson's Trichrome; Magnification = X 410





Figures 32 and 33: Transverse sections of fetal stem arteries from placenta in "toxemia" of pregnancy. Both vessels are filled with partially organized thrombus. There is neither proliferation of the smooth muscle, nor of endothelial cells.
Masson's Trichrome;
Magnification = X 256

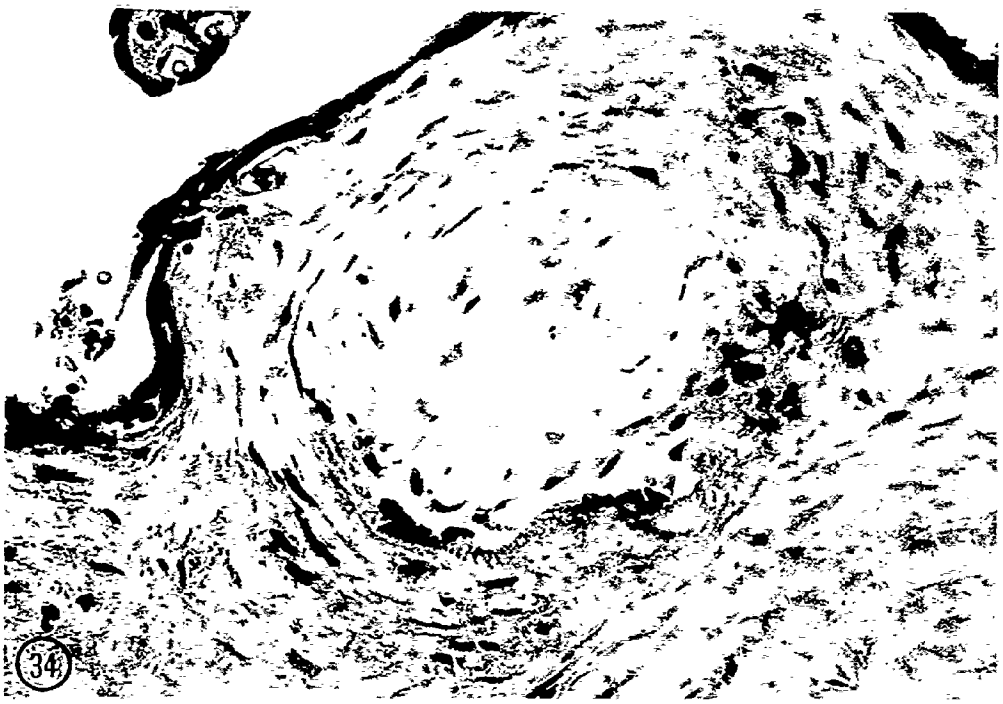
(32)



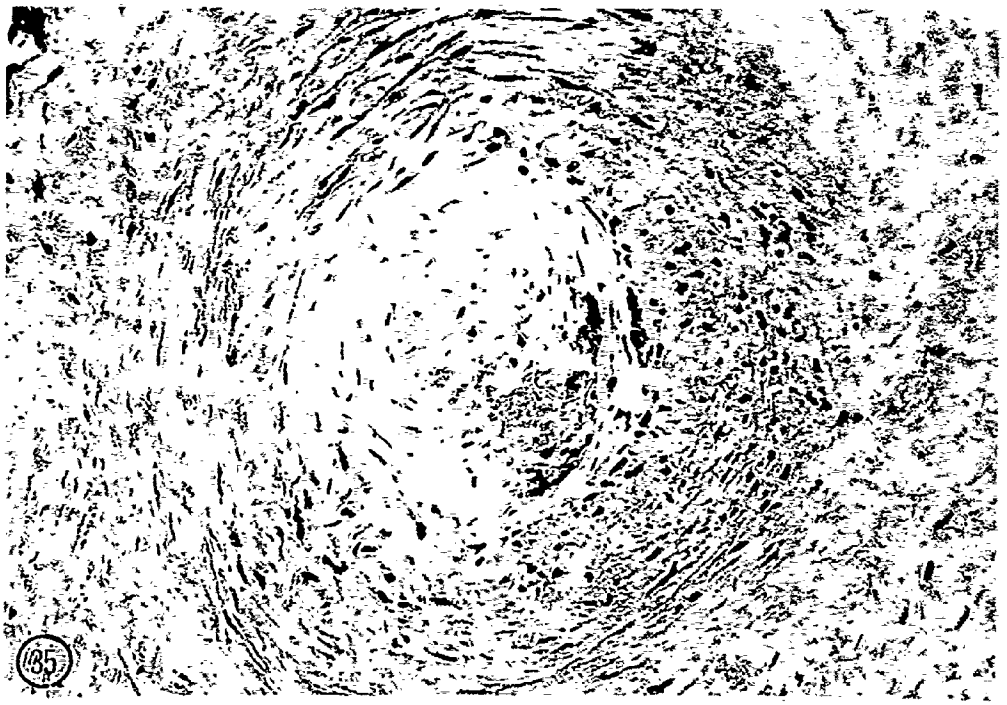
(33)

Figure 34: Photomicrograph of a transverse section of a fetal stem vein from a placenta of a "toxemic" patient to show a well organized thrombus as an excentric mass occluding the lumen of the vessel almost completely.
Masson's Trichrome; Magnification = X 256

Figure 35: Photomicrograph of a transverse section of a fetal stem artery of a placenta from a "toxemic" patient showing an almost entirely organized thrombus completely occluding the lumen. The artery shows a moderate proliferation of the smooth muscle cells.
Masson's Trichrome; Magnification = X 256



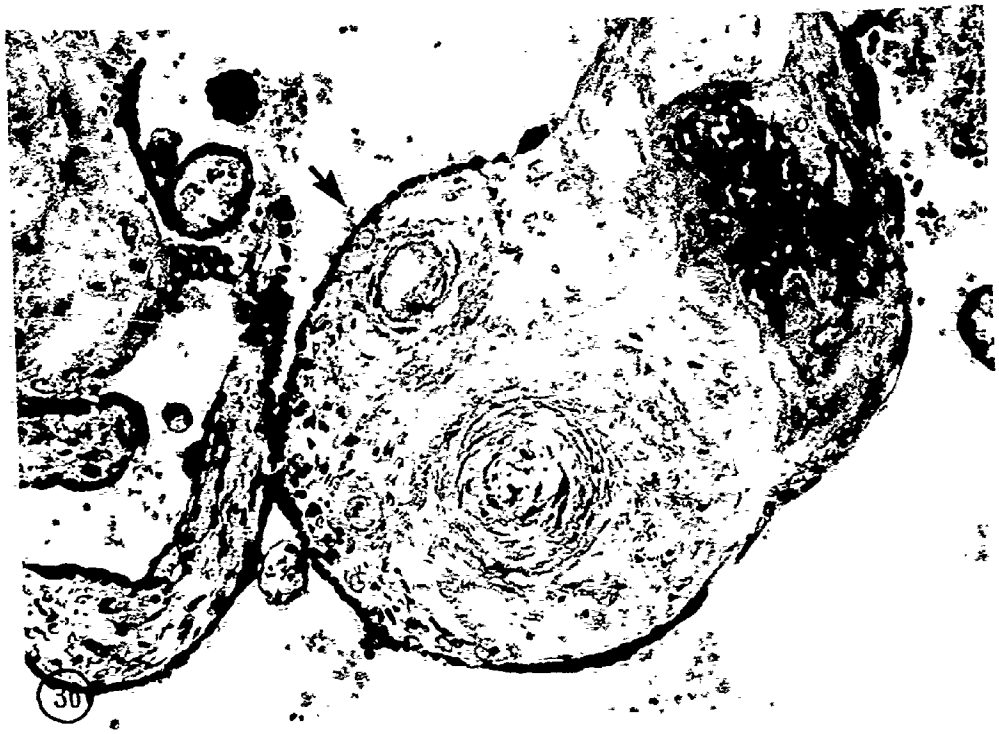
(34)



(35)

Figure 36: Photomicrograph of a transverse section of a vein in a ramus chorii to illustrate the occlusion of the lumen by "cushion" of acellular hyaline tissue in a placenta from a "toxemic" patient (arrow); it presumably represents an old organized and hyalinized thrombus.
Masson's Trichrome; Magnification = X 160

Figure 37: Photomicrograph of a transverse section of a fetal stem artery in a placenta from a "toxemic" patient showing the presence of cellular infiltration in the intima, media and adventitia disrupting the integrity of the arterial wall. No history of maternal or fetal infection was evident in this case.
Masson's Trichrome; Magnification = X 256


30

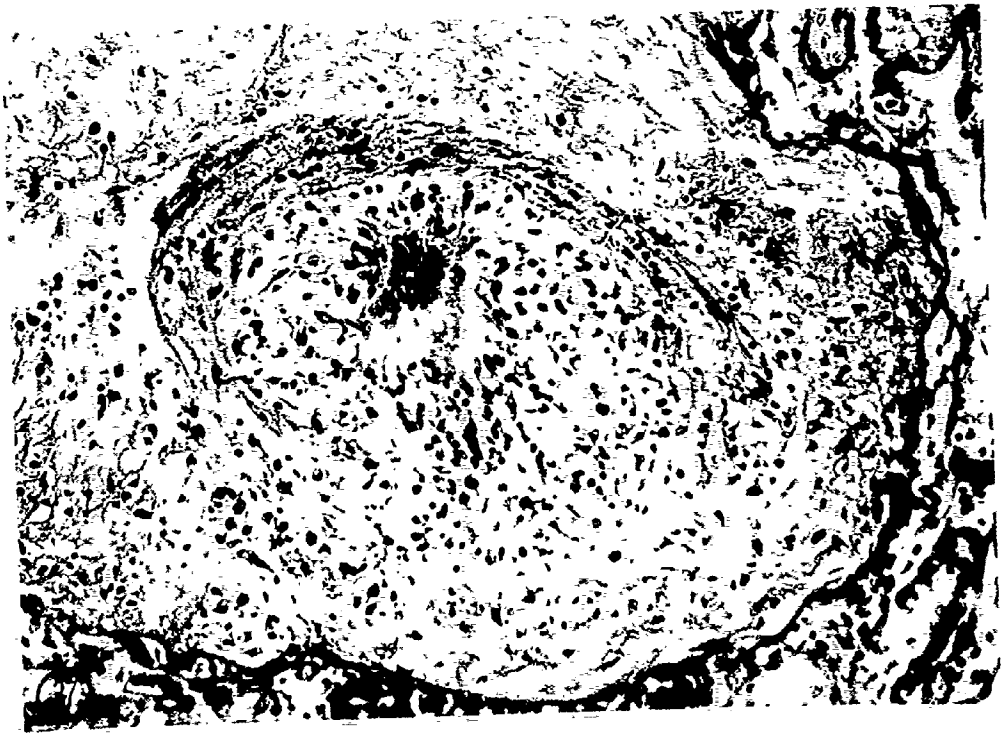
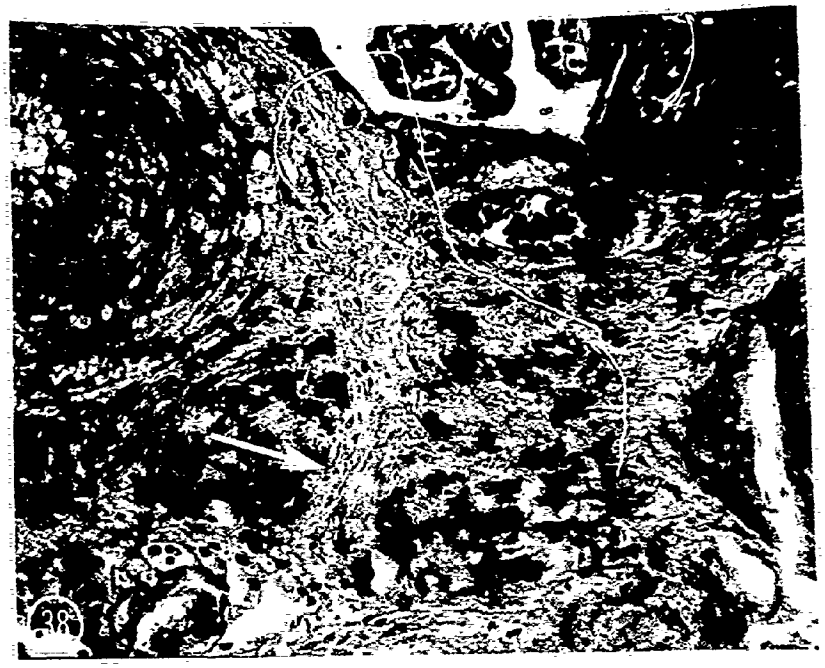


Figure 38: Colour photomicrograph of a section of a large villous stem in a placenta from a "toxemic" patient showing the orientation of the smooth muscle cells around and in between the fetal stem vessels. Some of the fibers are "connecting" one vessel with another in a sigmoidal fashion (arrow). Masson's Trichrome; Magnification = X 256

Figure 39: High power photomicrograph of a villous stem similar to that illustrated in Figure 38. It shows the conventional spindle shaped smooth muscle cells in the stroma of the villi connecting with fibers in the transitional area between themselves and the muscular coat of the fetal stem arteries.
Masson's Trichrome; Magnification = X 410


36


39

Figure 40: Detail of the intima and inner media of a fetal stem artery of 3rd order from a "toxemic" patient. The lumen (L) of the artery is extremely narrow. The endothelial cells (E) are proliferating towards and impinging upon the lumen. There is one cell in mitosis (M) with characteristics similar to those of mitotic endothelial cells (compare with Fig. 16). The media shows an increase in the number of smooth muscle cells. The interstitium is occupied by numerous bundles of collagen fibrils (C). Magnification = X 3,000.


E
L
E
M
SMC
SMC
C
40

Figure 41: Cross section of a fetal stem artery of 3rd order from a "toxemic" patient. The lumen is totally obliterated. The proliferating endothelial cells (E) are identified by their cellular features. The smooth muscle cells (SMC) of the medial coat are oriented circularly, tangentially or longitudinally, contributing by their proliferation to the thickening of the wall. In one area of the outer media there is "crowding" of longitudinal smooth muscle cells (arrows). Note numerous vacuoles (v) in the endothelial and medial areas.
Magnification = X 2,050.

41

Figure 42: Intima and media of a fetal stem artery of 3rd order in a "toxemic" patient. There is proliferation of endothelial cells (E) leading to a complete obliteration of the lumen. The smooth muscle cells (SMC) in the media are increased in number and have a distinctly circular arrangement (arrows).
Magnification = X 3,000.

42

Figure 43: Electron micrograph of an area of the intima and media of a fetal stem artery of 3rd order from a "toxemic" patient. The lumen is obliterated by proliferation of endothelial cells (E). The subendothelial space is occupied by smooth muscle cells (SMC), and basal membrane (BM). In the media, the smooth muscle cells are separated by widened interstitial spaces containing collagen fibrils (C). Magnification = X 3,000.


E
SMC
BM
SMC
C
43

Figure 44: Detail of the intima and innermost media of a fetal stem artery of 3rd order from a "toxemic" patient. Numerous endothelial cells (E) with distinct and less definitive cellular junctions (J) are seen proliferating towards the lumen (L); many of these are in close contact with smooth muscle cells (SMC). The collagen fibrils commonly found in the interstitial spaces of the media are not present in this area. Magnification = X 10,600.


E
L
E
SMC
E
J
44

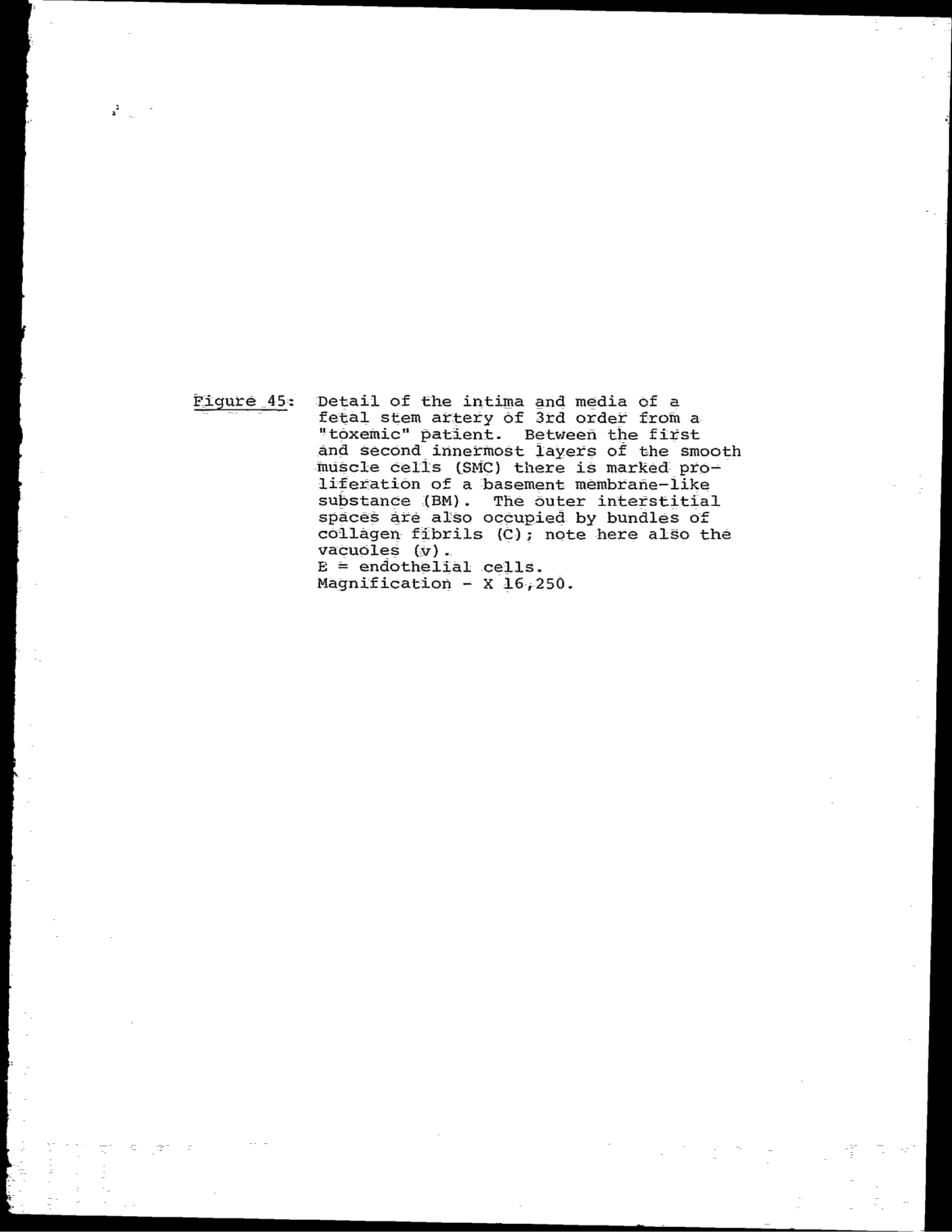
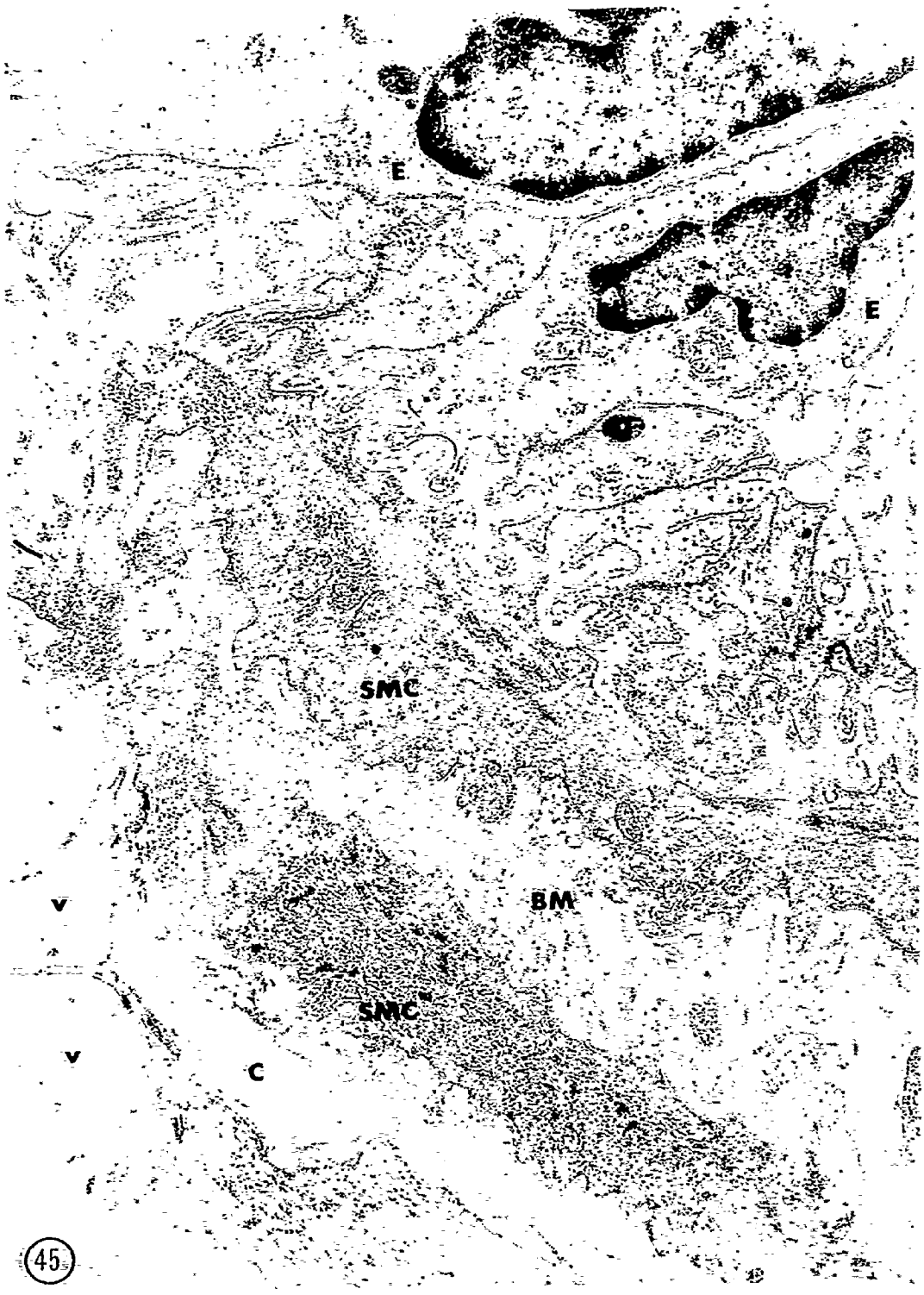
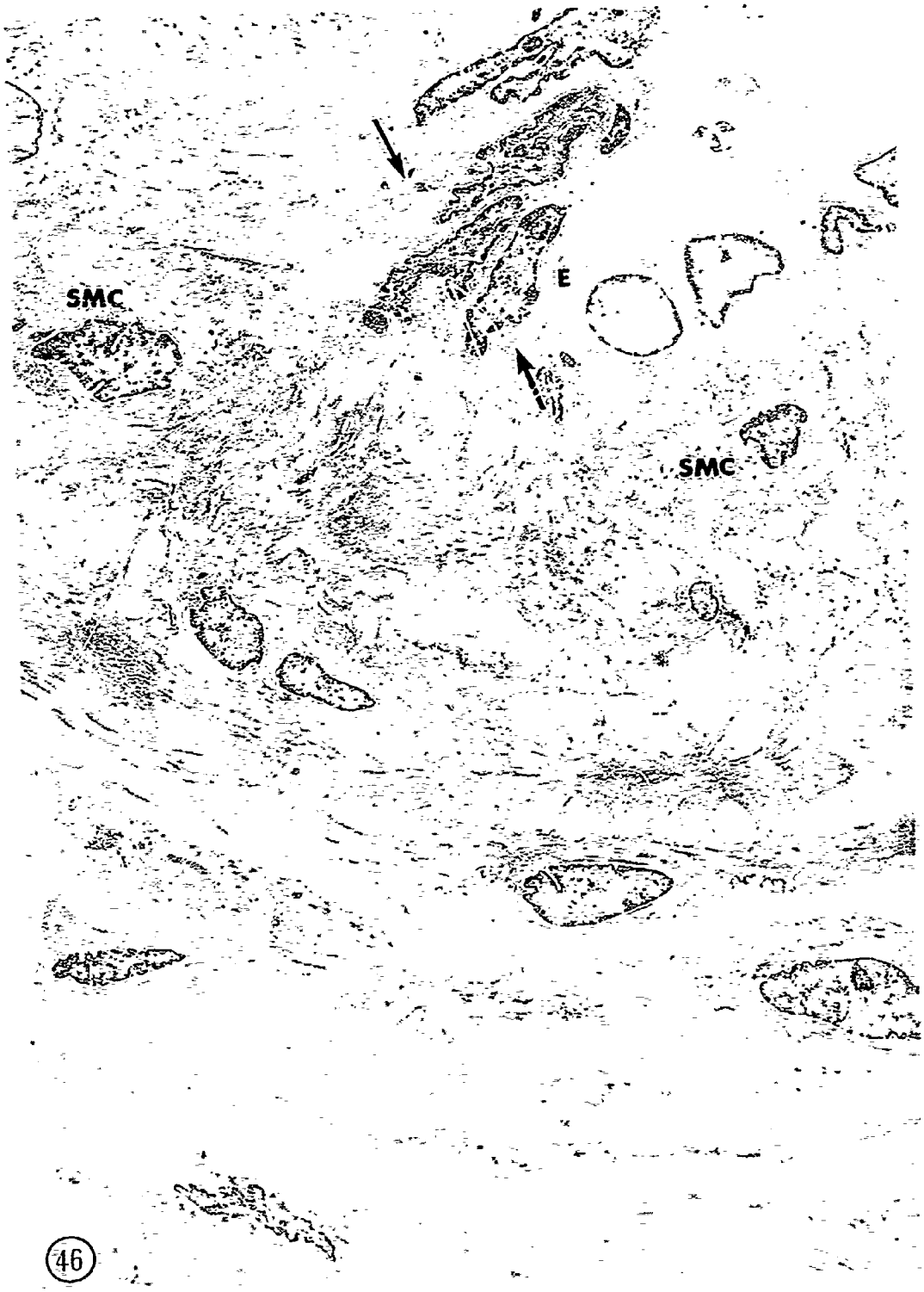


Figure 45: Detail of the intima and media of a fetal stem artery of 3rd order from a "toxemic" patient. Between the first and second innermost layers of the smooth muscle cells (SMC) there is marked proliferation of a basement membrane-like substance (BM). The outer interstitial spaces are also occupied by bundles of collagen fibrils (C); note here also the vacuoles (v).
E = endothelial cells.
Magnification - X 16,250.



45

Figure 46: Intima and media of a fetal stem artery of 3rd order from a "toxemic" patient. There is an increase in the number of medial smooth muscle cells (SMC) which appear to be in close contact with each other. The smooth muscle cells close to the lumen are longitudinally oriented; cells with certain features of SMC's appear with the endothelial cells (E) in the innermost arterial layer (arrows) and thus also impinging upon the lumen. Magnification = X 4,500.

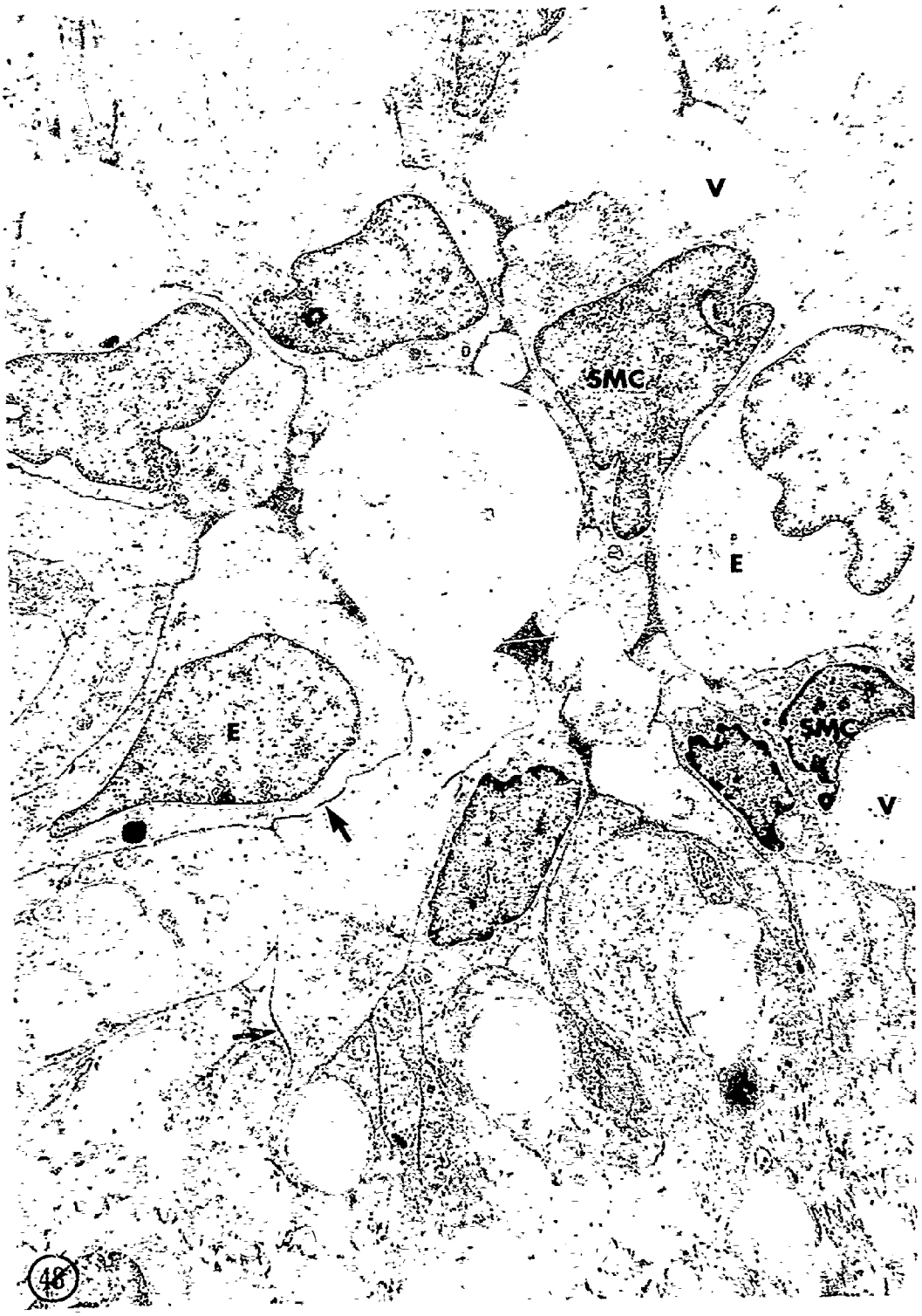


46

Figure 47: Detail of the intima of fetal stem artery of 3rd order from a "toxemic" patient. Some of the proliferating cells have unmistakable features of endothelial cells (E). Note the unequivocal cellular junctions (arrows) between them and the presence of Weibel-Palade (WP) bodies. Magnification = X 16,250.


E
WP
E
WP
E
WP
47

Figure 48: Electron micrograph of the intima of fetal stem artery of 3rd order from a "toxemic" patient. The lumen is obliterated by the proliferation of endothelial cells (E). There are extensive membrane appositions between the endothelial cells and at several points they are forming cellular junctions (arrows). Numerous vacuoles (V) are membrane bounded and represent swollen processes of smooth muscle cells (SMC). Magnification ≈ X 7,250.


V
SMC
E
E
SMC
V
46

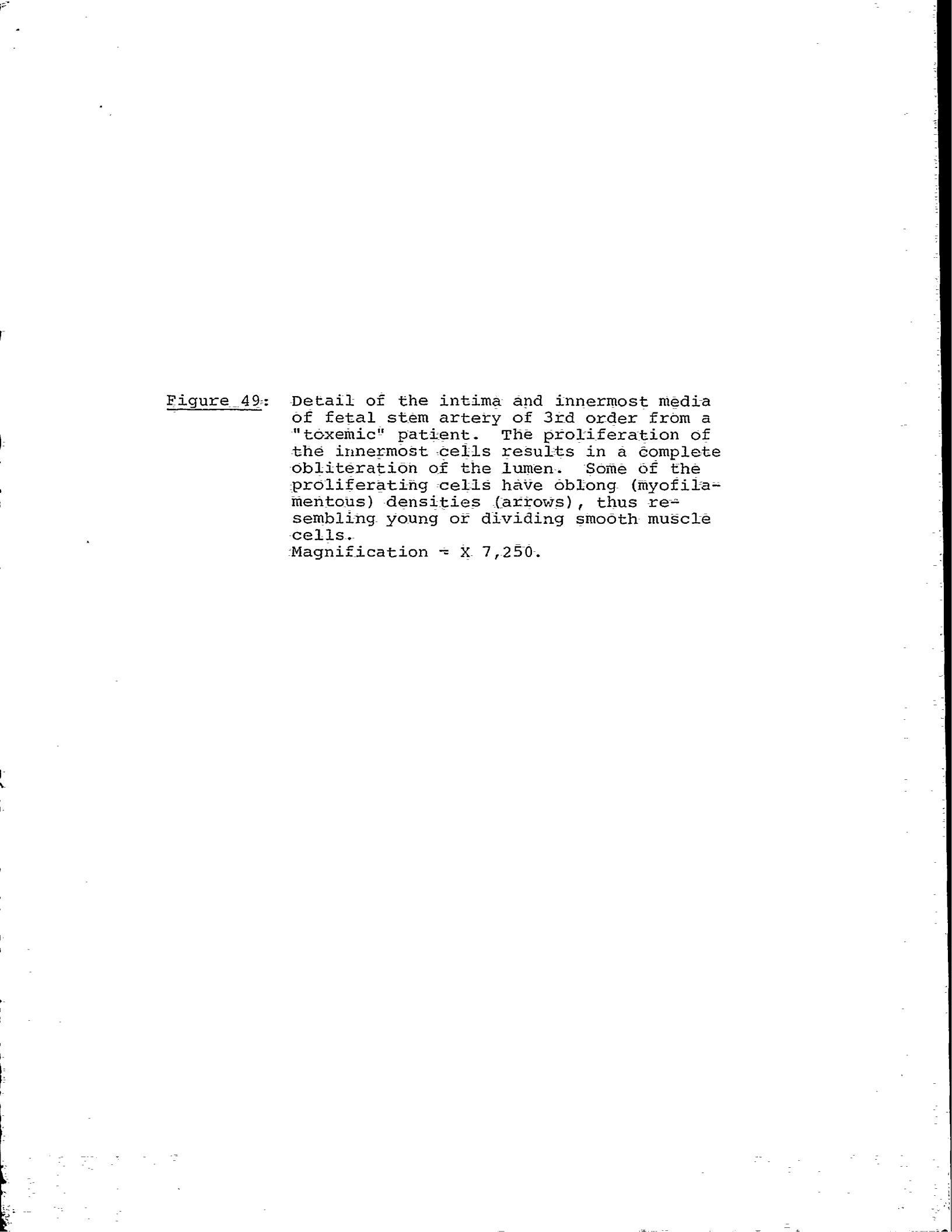
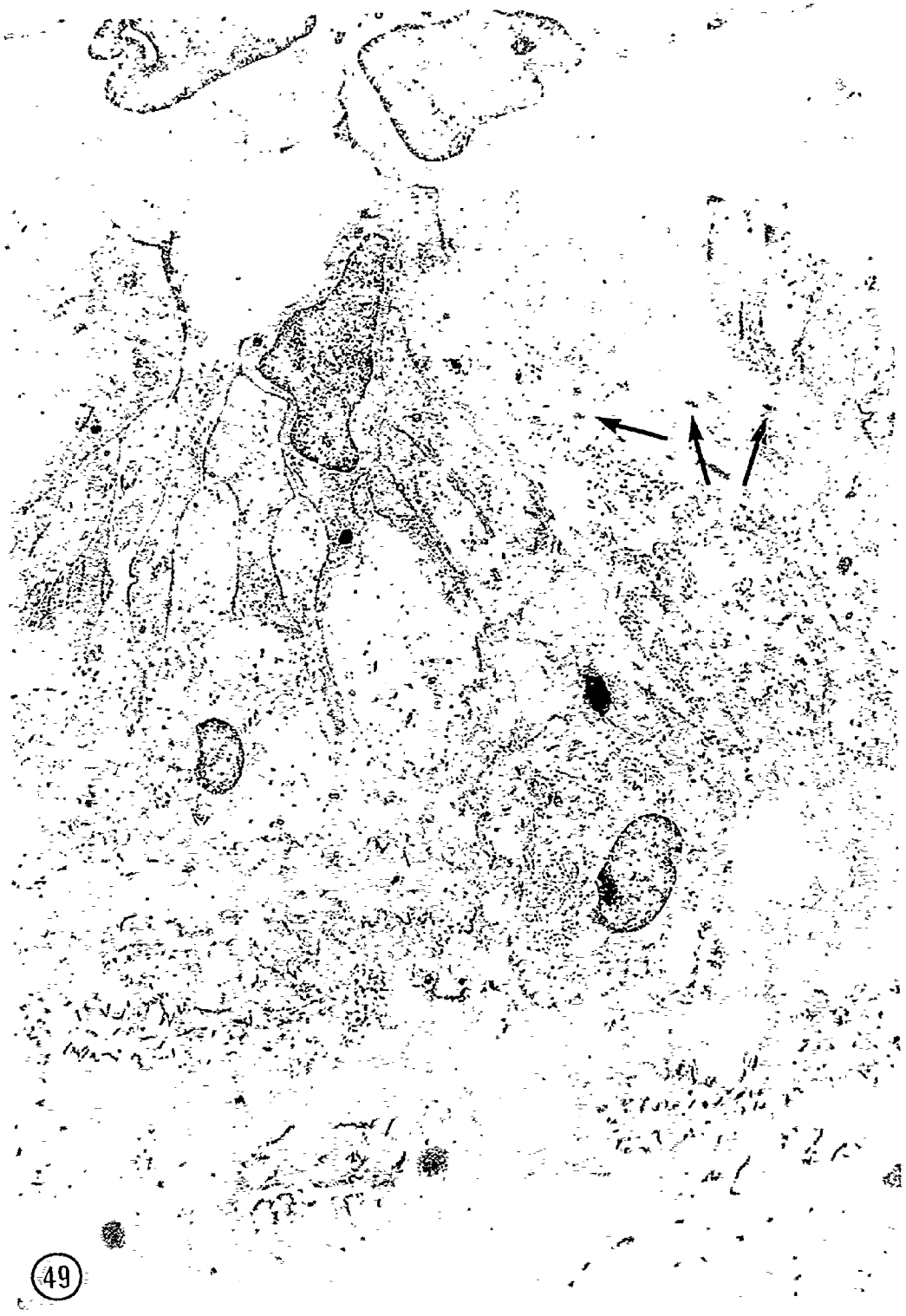


Figure 49: Detail of the intima and innermost media of fetal stem artery of 3rd order from a "toxemic" patient. The proliferation of the innermost cells results in a complete obliteration of the lumen. Some of the proliferating cells have oblong (myofilamentous) densities (arrows), thus resembling young or dividing smooth muscle cells.
Magnification = X 7,250.

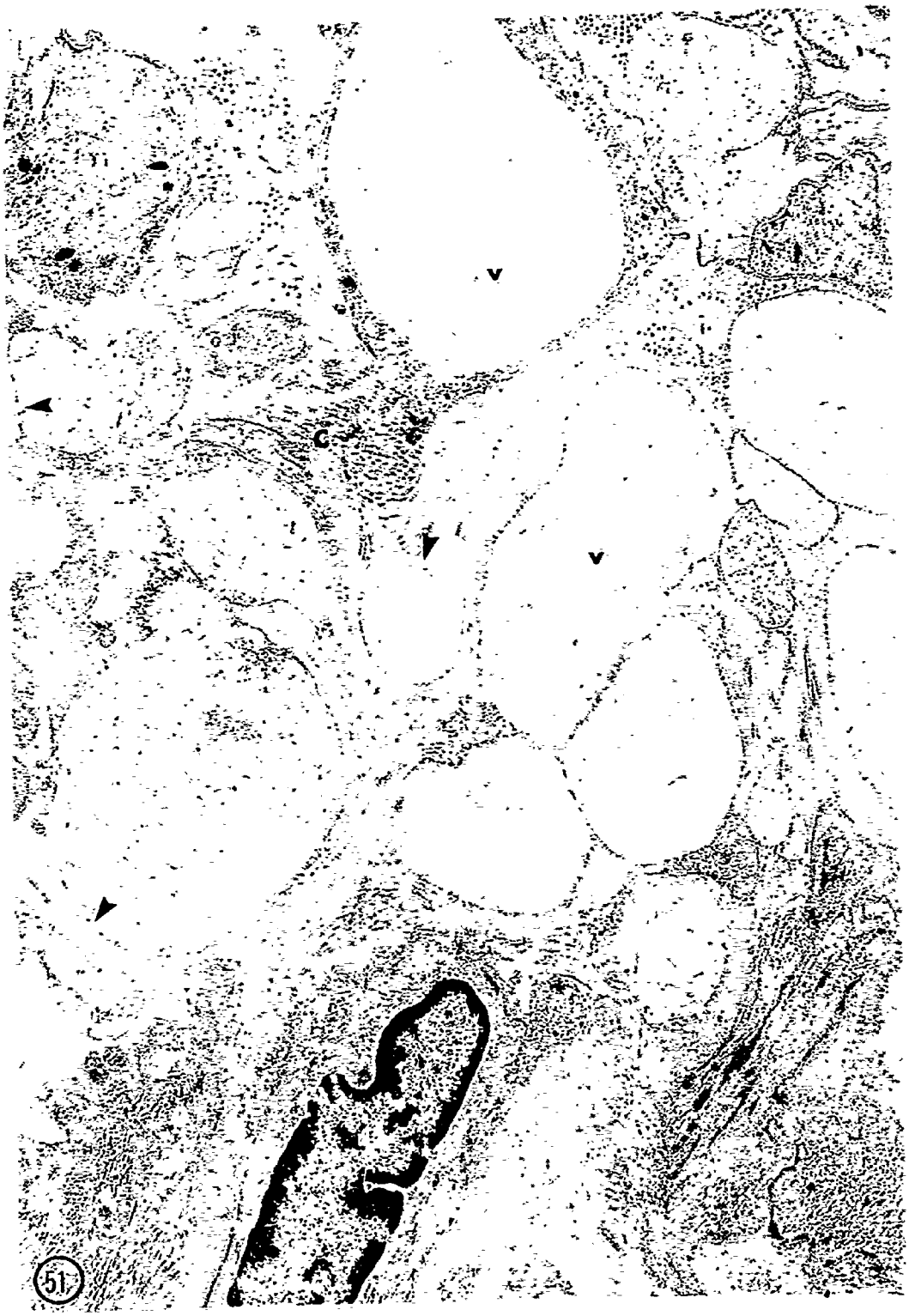


(49)

Figure 50: Detail of the intima of fetal stem artery of 3rd order from a "toxemic" patient. One of the cells, with features reminiscent of endothelial cells, is in mitosis (M). Some of the subendothelial smooth muscle cells (SMC) show the presence of centriolar complex (cen). The collagen (C) fibrils are increased in the interstitium.
Magnification = X 8,750.


M
SMC
SMC
cen
C
50

Figure 51: The media of a fetal stem artery of 3rd order from a "toxemic" patient. Most of the vacuoles (v) are entirely membrane bounded and represent swollen cellular processes of smooth muscle cells. Occasionally, a portion of that membrane is missing (arrows). The interstitial spaces appear widened and contain randomly oriented bundles of collagen fibrils (C).
Magnification = X 13,250.


v
v
51

APPENDIX A

ANALYSIS OF VARIANCE

Key- Sprout = Average Sprout Count in all Areas
Preg = Pregnancy Type
Mat = Maternal Age
Gest = Gestation
Placen = Placental Weight
Birth = Birth Weight
Apgar = Apgar Score

SOURCE OF VARIATION	SUM OF SQUARES	DF	F	SIGNIF OF F
Covariates	630.746	5	126.149	.001
Mat	26.849	1	26.849	.042
Gest	.991	1	.991	.999
Placen	.231	1	.213	.999
Birth	13.643	1	13.643	.147
Apgar	347.260	1	347.260	.001
Main Effects	5008.151	2	2504.076	.001
Preg	5008.151	2	2504.076	.001
Residual	930.840	142	6.555	
Total	6569.737	149	44.092	

150 Cases were processed.
0 Cases (0 PCT) were missing.

STANDARDIZED REGRESSION COEFFICIENTS

Covariate	Beta
MAT	-.097
GEST	-.059
PLACEN	-.001
BIRTH	-.001
APGAR	-1.163

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
Covariates	.076	5	.015	2.344	.044
Mat	.029	1	.029	4.420	.035
Gest	.011	1	.011	1.631	.201
Placen	.004	1	.004	.585	.999
Birth	.017	1	.017	2.566	.107
Apgar	.026	1	.026	3.963	.046
Main Effects	.361	2	.180	27.922	.001
Preg	.361	2	.180	27.922	.001
Residual	.917	142	.006		
Total	1.353	149	.009		

STANDARDIZED REGRESSION COEFFICIENTS

Covariate	Beta
MAT	.003
GEST	.006
PLACEN	.000
BIRTH	-.000
APGAR	.010

APPENDIX B

ANALYSIS OF VARIANCE

LUMEN/WHOLE RATIOS

PT = Pregnancy Type

SOURCE OF VARIATION	D.F.	SUM OF SQUARES	MEAN SQUARE	RATIO	VALUE
Between Cases	149	4.060			
PT	2	1.146		28.65	<.001
Cases/PT	147	2.914	.020		
Within Cases	300	1.719			
Area	2	.831	.416	138.50	<.001
Area x PT	4	.042	.011	3.50	.005<p<.01
Area x (Cases/PT)	294	.846	.003		
TOTAL	449	5.779			

SPROUT COUNTS

SOURCE OF VARIATION	D.F.	SUM OF SQUARES	MEAN SQUARE	F RATIO	P VALUE
Between Cases	149	19709.21			
Preg. Type (PT)	2	16538.94	8269.47	383.39	<.001
Cases/Pt	147	3170.27	21.57		
Within Cases	300	3498.67			
Area	2	2894.01	1447.01	871.69	<.001
Area x PT	4	117.32	29.33	17.67	<.001
Area x (Cases/PT)	294	487.34	1.66		
TOTAL	449	23207.88			